

水産総合研究センター 研究報告

20

平成19年2月

BULLETIN OF FISHERIES RESEARCH AGENCY

No. 20
February 2007

Proceedings of the International Workshop on Spiny Lobster Seed Production
Technology, Mie, Japan, November 24-25, 2005

Library
Pacific Islands Fisheries Science Center
Nat'l Marine Fisheries Service
2570 Dole St. Honolulu, HI 96822-2396
Received  May 2007

独立行政法人 水産総合研究センター
神奈川県横浜市
FISHERIES RESEARCH AGENCY
Yokohama, Kanagawa, Japan

水産総合研究センター研究報告

Bulletin of Fisheries Research Agency (No. 20)

Proceedings of the International Workshop on Spiny Lobster Seed Production
Technology, Mie, Japan, November 24-25, 2005

Editors of Special Issue:

Hideaki Aono, Satoshi Mikami and Masahito Yokoyama

Published by Fisheries Research Agency

Queen's Tower B 15F, 2-3-3, Minato Mirai, Nishi-ku, Yokohama, Kanagawa 220-6115 Japan

President of FRA: Kyoichi Kawaguchi

Web site: <http://www.fra.affrc.go.jp>

Printed by Nisshoprinting.co

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ISSN 1346-9894

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Proceedings of the International Workshop on Spiny Lobster Seed Production Technology, Mie, Japan, November 24-25, 2005

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Opening Remarks

Good morning, everyone. It is my pleasure to greet you on behalf of National Research Institute of Aquaculture. To all of you present here, I take pleasure in expressing my sincere thanks for your attendance at this workshop in spite of your busy schedules. My special thanks go to those who have traveled a great distance to be here from Australia and New Zealand. I should also like to thank Dr. Satoshi Mikami for his assistance with inviting the foreign team.

As you know, aquaculture has been popular all over the world and the recent progress of aquaculture technology is incredible.

But there still remain many subjects which need to be overcome. Seed production is one of those issues. We have not been able to produce seeds of many kinds of fish species for aquaculture, though they are popular species. Eel and spiny lobster are such species.

There are many difficulties to produce seedlings artificially, because of the novel problems associated with the ecological and physiological characteristics of the larvae of these species.

To promote seed production technology of these two species, the Ministry of Agriculture, Forestry and Fisheries of Japan has just initiated a major national research program entitled "Development of seed production technology in Japanese eel and Japanese spiny lobster" this year. We are now working to develop this research program.

The purpose of this workshop is to discuss recent progress in the fields of spiny lobster aquaculture, especially technological improvements necessary to increase the survival rate during the stage of phyllosoma. We have five speakers from Australia and New Zealand, and seven from Japan. I hope this international workshop will both encourage international cooperation and stimulate advances in these fields.

By the way, this is the best season for sightseeing here and the Ise area is one of the most important historic places in Japan. Ise Shrine has more than 1,300 years of history. I hope that whilst you are here you can experience some aspects of Japanese culture and traditions and of course, I hope you can try some Japanese food, too.

I wish you every success with your research and hope that you all find this workshop beneficial and it helps stimulate you to future achievements. I also hope that you will be able to enjoy your stay in Japan.

Thank you.

Dr. Yasuji Sakai
Director General
National Research Institute of Aquaculture



*International Workshop on Spiny Lobster
Seed Production Technology*

*National Research Institute of Aquaculture,
Fisheries Research Agency*

November 24-25, 2005

Eukaryotes from the hepatopancreas of lobster phyllosoma larvae

Nobuaki SUZUKI^{*1,5}, Keisuke MURAKAMI^{*2}, Haruko TAKEYAMA^{*3} and Seinen CHOW^{*4}

Abstract In order to investigate unknown prey organisms for lobster phyllosoma larvae, molecular approach was developed and adopted using the laboratory-reared and wild phyllosoma samples. The central domain of 18S rDNA was amplified via nested PCR using crude DNAs extracted from the hepatopancrea of the larvae and cloned into plasmid. Clones having 18S rDNA different from the hosts were screened by RFLP analysis, and the nucleotide sequences determined were subjected to FASTA homology search and phylogenetic analysis to identify the source of eukaryotic organisms. Feasibility of this method was confirmed in the laboratory-reared larvae of the Japanese spiny lobster (*Panulirus japonicus*) fed exclusively on either gonad of common mussel (*Mytilus edulis*) or *Artemia* nauplii. From the two wild-caught larvae (*Scyllarus* sp. and *P. japonicus*), 178 and 168 clones were isolated, of which 132 and 4 clones had different restriction profiles from the hosts, respectively. DNAs of Cnidaria and Urochordata were observed from the scyllarid larva, while those of fungi and host variant were observed in *P. japonicus* larva.

Key words: prey organisms, phyllosoma larvae, *Panulirus*, scyllarid, 18SrDNA

Lobsters are very important fishery resources. Their larvae called as phyllosoma have the long planktonic period extending from several months to more than a year (Chittleborough and Thomas, 1969; Lesser, 1978; Kittaka, 1988). Although predator-prey interaction may be very important information to understanding larval ecology under dynamics of food web, the prey organisms of the phyllosoma in natural environment are not well known. In scyllarid phyllosoma larvae, a unique behavior as riding on jelly fish, called "piggyback riding", has been observed and expected as a means of transport with meals (Shojima, 1963; Thomas, 1963). Laboratory experiments offering a variety of planktons indicated that phyllosoma larvae of *Panulirus interruptus* and *Jasus edwardsii* preferred medusae, ctenophores, chaetognaths and other soft-bodied animals (Mitchell, 1971; Kittaka, 1994). However, the phyllosoma larvae may change prey organisms as

they grow and disperse into very wide range during the long larval period. Unlike the other Decapod crustacean larvae, laboratory observation indicated that phyllosoma larvae may be a sucking predator (Murakami, personal communication). Observations on developments in the mouthpart suggested that early-stage phyllosoma of *J. edwardsii* would benefit from a diet comprising soft gelatinous zooplankton (Johnston and Ritar, 2001).

Although rearing the phyllosoma larvae has been considerably difficult, successful metamorphoses have been achieved in several Palinurid species by feeding *Artemia* nauplii supplemented with gonad of common mussel (*Mytilus edulis*) (Kittaka, 1994; Kittaka, 1997; Kittaka *et al.*, 1997). Yet, the survival rate is still very low. Thus, the information of natural diets for phyllosoma larvae may be very important to prepare their food for aquaculture.

Recently, DNA-based approaches to reveal prey

Received: November 25, 2005

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organisms have been applied to the gut contents of several marine organisms (Asahida *et al.*, 1997; Jarman *et al.*, 2002; Rosel and Kocher, 2002; Saitoh *et al.*, 2003; Jarman *et al.*, 2004; Blankenship and Yayanos, 2005). These studies, however, dealt with adult predator and/or used PCR primers designed for suspicious or target prey organisms. In order to investigate unknown prey organisms of the lobster phyllosoma larvae, we have designed universal primers for amplifying relatively variable central domain of 18S rDNA, and attempted to develop detection technique of foreign DNAs from an alimentary canal. Here, we introduce the technique and report the preliminary results of our attempts.

Materials and Methods

Phyllosoma specimens and DNA extraction

Six phyllosoma larvae (five of palinurid and one of scyllarid lobsters) were used in this study (Table 1). The developmental stages of these phyllosoma larvae were determined according to Inoue (1981), Matsuda and Yamakawa (2000), and Johnson (1971a, b). Four larvae were of laboratory-reared Japanese spiny lobster, *P. japonicus*, hatched on July 2002 and kept at the Minamiizu Station, National Center for Stock Enhancement, Japan. The phyllosoma larvae were divided into two feeding conditions; one exclusively fed common mussel (*Mytilus edulis*) gonads (Myt) and the other fed *Artemia* nauplii (Art). Two wild-caught phyllosoma larvae were from the Pacific (Pac-1 and -2) through research cruises in 2002 and 2003. The specimens were frozen immediately after sampling, transferred to the laboratory and preserved in ethanol. Prior nucleotide sequence analysis on the mitochondrial COI gene

(data not shown) indicated that one (Pac-2) was of *P. japonicus* but the other (Pac-1) showed poor identity for the scyllarid species because of the small number of reference sequences in the DNA data base. Consequently, this larva was morphologically assigned to *Scyllarus* sp. according to Johnson (1971a, b).

Prior to DNA extraction, the surface of the phyllosoma larvae was washed well with sterile distilled water to remove possible contaminations from the surface. Hepatopancreas tissues with the contents were pipetted out from small incision made in the cephalic region. This "hepatopancreas content" and a part of pereopod were homogenized using tephlon pestle followed by crude DNA extraction using standard proteinase K digestion /phenol-chloroform extraction. The washed water from the wild phyllosoma larvae was recovered, ethanol-precipitated and used as a negative control.

PCR primers to amplify 18S rDNA

Two sets of universal primers were designed to amplify the central domain of 18S rDNA for nested PCR. Nucleotide sequences of the primers are available by connecting with the present authors. The priming regions were highly conserved among eukaryotic organisms to the extent that both primer combinations could amplify homologous regions of diatom (*Chaetoceros* sp.), lobster (*P. japonicus*), eel (*Anguilla japonica*) and human (*Homo sapiense*) (data not shown). The PCR reaction mixture contained 0.2 U Taq DNA polymerase (Applied Biosystems Inc.), 0.2 mM of each dNTP, 1.0 μ l 10 x buffer with 15 mM MgCl₂ (Applied Biosystems Inc.), 10 pmol of each primer, and 10 to 50 ng template DNA. The final volume of the reaction mixture was

Table 1. Samples of lobster phyllosoma larvae used in this study

Source or locality	Family	Species	N	BL (mm)	stage	ID
Laboratory-reared						
Aremia feeding	Palinuridae	<i>Panulirus japonicus</i>	2	9.4, 10.6	VII	Art
Mytilus feeding	Palinuridae	<i>Panulirus japonicus</i>	2	9.0, 9.4	VII	Myt
Wild-caught						
Pacific	Scyllaridae	<i>Scyllarus</i> sp.	1	19.3	VII?	Pac-1
	Palinuridae	<i>Panulirus japonicus</i>	1	11.5	VII	Pac-2

N : number of specimens, BL : body length

made up to 10 μ l with sterile distilled water. PCR was carried out with an initial denaturation at 95 $^{\circ}$ C for 2 min, followed by 30 cycles of amplification (denaturation at 95 $^{\circ}$ C for 30 sec, annealing at 55 $^{\circ}$ C for 30 sec, and extension at 72 $^{\circ}$ C for 1.5 min, with a final extension at 72 $^{\circ}$ C for 10 min). The first and second PCR products were electrophoresed through a 1.5-2.0 % agarose gel in TBE buffer (50 mM Tris, 1 mM EDTA, and 48.5 mM boric acid) to confirm amplification.

Cloning, screening and sequencing of amplified fragments

Amplicons from the second PCR were directly subjected to TA cloning without further purification. Cloning was carried out using pGEM[®]-T Easy Vector System I (Promega). Transformed clones were first screened by colony-direct PCR with a primer set used for the second PCR. Since preliminary restriction assay indicated three endonucleases (*Alu* I, *Hpy*188I and *Mse* I) to have multiple restriction sites in the phyllosoma larvae, the PCR products were digested with these enzymes. Digested samples were electrophoresed on an agarose gel to select clones having restriction profiles distinct from those of the hosts. One or a few PCR product from each restriction type was selected arbitrarily and purified using ExoSAP-IT (Amersham). Sequencing reaction was carried out using the BigDye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems Inc.) with the forward primer for the second, and nucleotide sequences were determined on an automated DNA sequencer (ABI 310, Applied Biosystems Inc.).

Homology search and sequence analysis

DNA sequences obtained were subjected to homology search using FASTA algorithm through DDBJ (DNA Data Bank of Japan) to investigate sequence similarities among organisms. Highly identical sequences nominated by FASTA (basically >95 % identity score) were selected and incorporated into phylogenetic analysis. Alignments of DNA sequences were performed with ClustalW algorithm (Thompson *et al.*, 1994) followed by manual editing. The aligned sequences were imported into PAUP* (Swofford, 2000) to infer positions of the cloned DNAs on neighbor-joining (NJ) tree. Genetic distances among sequences were calculated under Kimura's two parameter (K2P) substitution model with complete deletion option. Bootstrap probabilities with 1,000 replications were calculated to assess reliability on each node of NJ tree.

Results and Discussion

Detection of dietary DNA using laboratory-reared phyllosomas

The target region of 18S rDNA was well amplified in all laboratory-reared larvae by the nested PCR. Results of the experiment are summarized in Table 2. Individuals in each feeding condition were considered together. Transformation efficiency was high for both samples (>80 %). Of 125 white colonies in Art and 108 in Myt, subjected to colony direct PCR using the second PCR primers, amplicons were obtained in 116 and 91 colonies, respectively.

In the sample fed on *Artemia* nauplii (Art), a total of five clones had restriction profiles inconsistent from the host. FASTA search indicated that the

Table 2. Results of feed DNA detection using laboratory - reared phyllosoma larvae

Sample ID	No. of amplification/PCR	RFLP profile		No. of types	Identified eukaryote (no. of clones)	
		host	non-host			
Art	116/125	111	5	4	<i>Artemia</i>	(1)
					unidentified	(3)
					<i>Panulirus</i> *	(1)
Myt	91/108	55	36	8	<i>Mytilus</i>	(14)
					<i>Panulirus</i> *	(5)
					<i>Cryptomeria</i>	(15)
					unidentified	(2)

*Supposed to be host variants

insert of one clone appeared to be of *Artemia salina* (99 % nucleotide identity). Three clones were unidentified because of the low nucleotide identity (<85 %). The last clone had similar 18S rDNA sequence with *Panulirus* species with high identity (>95 %) and determined as a host variant.

In the sample fed on gonad of common muscle (Myt), 36 clones with 8 restriction types were observed to have restriction profiles inconsistent from the host. A total of 14 clones were determined to be *Mytilus* 18S rDNA, whereas 15 clones were apparent contaminants of terrestrial plant, *Cryptomeria japonica* (Japanese cedar), five being host variants. The other two clones were unidentified because of the low identity (80 %).

The results obtained in the laboratory-reared samples clearly indicate that the experimental procedure presented in this study is powerful enough to detect DNAs of fed organisms in the hepatopancreas of lobster phyllosoma larvae. The detecting ability for dietary DNA may depend on the amount of tissues consumed (Wallace, 2004; Deagle *et al.*, 2005) and also on a degree of digestion. Better detection observed in the Myt sample could be caused by the difference in amount of consumed either *Mytilus* gonad or *Artemia* nauplii. Actually, cephalic region of phyllosoma fed on *Mytilus* gonad was yellowish, while that of *Artemia* was transparent. Validation of detecting ability for quality in consumed food will be expected in future.

Analysis of wild-caught phyllosoma larvae

Results of experiment using hepatopancreas of wild-caught phyllosoma larvae are summarized in Table 3. Little amplification was observed from washing water samples, and hence, indicated

that cross contamination from the body surface of phyllosoma larvae was very little. From the hepatopancreas contents each of Pac-1 and Pac-2, 178 and 168 clones were obtained, respectively. Of all the clones derived from the Pac-1, 132 clones (74 %) showed restriction profiles distinct from the host, in contrast to only four clones (2.4 %) doing in the Pac-2. Based on the RFLP analysis, eight and two restriction types were distinguished from the host in Pac-1 and Pac-2, respectively.

The nucleotide sequence alignment among hepatopancreas-derived and FASTA-nominated sequences revealed eight clones in Pac-1 and three in Pac-2 to be chimeric artifacts between partial sequences of fungus and host 18S rDNA. These chimera sequences were excluded from the phylogenetic analysis. One restriction type in Pac-1, including 10 clones, was not identified because of difficulty for sequencing.

The FASTA searches and phylogenetic analysis arranged six sequences from Pac-1 and one from Pac-2 on the unrooted NJ tree among possible organism nominated in FASTA (Fig. 1). Two animal taxa (phylum Cnidaria and subphylum Urochordata) and one plant family (Pinaceae) were clearly assembled with 100 % bootstrap probabilities. All clonal sequences obtained from Pac-1 were fallen into the animal clade, whereas one sequence from Pac-2 was into the opposite plant clade, suggested to be Ryukyu island pine by FASTA. This pine DNA may be introduced into the laboratory environment from airborne pollen. Such is the case with the Japanese cedar observed in the laboratory-reared phyllosoma larvae. Of the sequences fallen into the cnidarian clade, one (Pac-1 Cnidaria1) was assigned to Scyphozoa and the other two (Pac-1 Cnidaria2

Table 3. Results of eukaryotes DNA detection using wild-caught phyllosoma larvae

Specimen ID	No. of amplicons/PCR	RFLP profile		No. of types*	Identified eukaryote ^{No. of types}	
		host	non-host		(No. of clones)	
Pac-1	178/192	46	132	8	Urochordata ³	(55)
					Cnidaria ³	(59)
					chimera ¹	(8)
					unidentified ¹	(10)
Pac-2	168/184	164	4	2	pine tree ¹	(1)
					chimera ¹	(3)

*Host type excluded

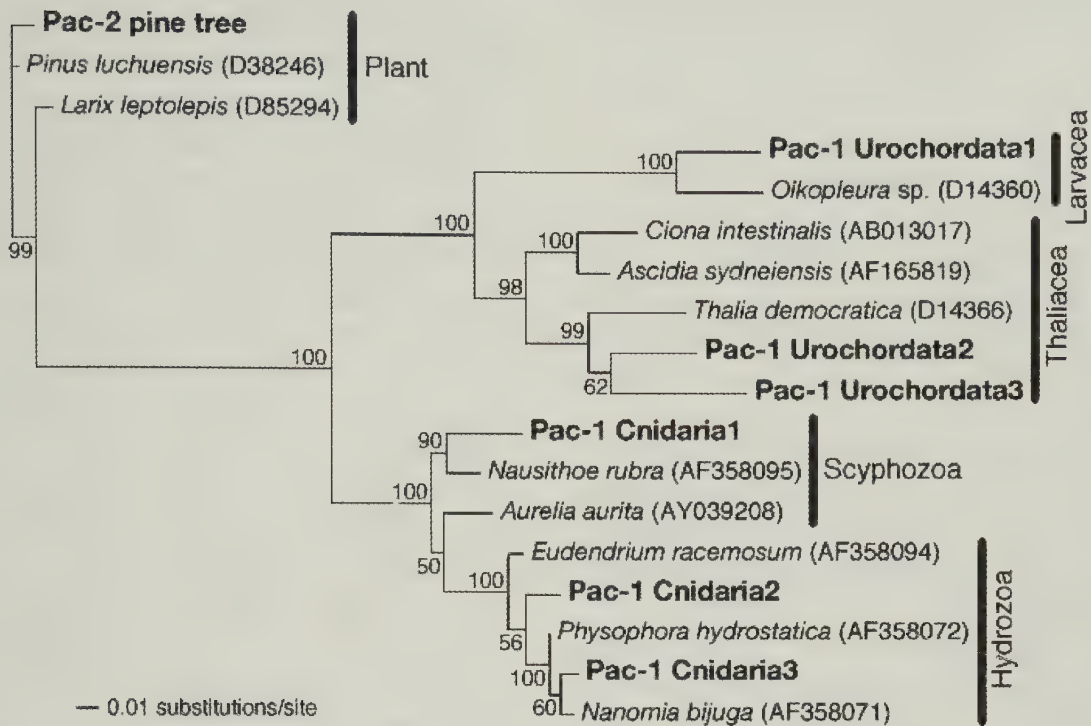


Fig. 1. Neighbor-joining tree for affiliating 18S rDNA sequences obtained from the wild phyllosoma hepatopancreas. Sequences from the hepatopancreas were shown in bold host ID and eukaryote 18S rDNAs nominated by FASTA search were in italic with the accession No. in parenthesis. Numbers on branches indicate bootstrap probabilities with 1,000 replications.

and 3) to Hydrozoa. In the Urochordata clade, one sequence (Pac-1 Urochordata1) was assigned to Larvacea and the other two (Pac-1 Urochordata2 and 3) to Thaliadea.

In the present analysis for wild-caught phyllosoma larvae, Cnidaria and Urochordata are likely to be prey organisms at least for the scyllarid phyllosoma Pac-1. This corresponds to the observation for nematocysts in the fecal mass of a scyllarid phyllosoma (Sims and Brown, 1968). Alternatively, no clones derived from possible prey were detected from the Japanese spiny lobster larva Pac-2. This discordance might also be caused by the difference in the amount of consumed food and/or degree of digestion like as the case of laboratory-reared larvae. In the reared phyllosoma sample fed *Artemia* nauplii (Art), only one clone of *Artemia* feed could be found in a total of 116 amplicons through colony-direct PCR, in spite of sufficient feeding and immediate

sampling after meal. More clones with much experimental effort might have produce detection for wild-caught larvae. It should be consider how effort detects effectively foreign DNAs derived from possible prey organisms.

Acknowledgements

We would like to thank T. Yoshimura, Seikai National Fisheries Research Institute, for providing valuable information on taxonomy and ecology of lobster larvae. We also like to thank K. Satoh, National Research Institute of Far Seas Fisheries, and the members of RV Shunyo-Marui for assistance in sample collection, and H. Hasegawa and M. Michibayashi, for their superb technical assistance in DNA analyses. This work was supported in part by grants from the Japanese Society for the Promotion of Science, the Ministry of Agriculture, Forestry, and

Fisheries of Japan, and a Grant-in-Aid for Scientific Research on Priority Areas (B) (No. 15380137) from the Ministry of Education, Science, Sports and Culture.

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Revealing the natural diet of the phyllosoma larvae of spiny lobster

Andrew JEFFS *

Abstract An important bottleneck in the development of aquaculture of spiny lobsters is larval feeding and nutrition. Researchers have used a wide variety of natural foods and artificial food materials in attempts to improve the larval culture of these species. Unfortunately, very little is known about the natural feeding ecology and diet of spiny lobster larvae or phyllosomes. This paper reviews research undertaken in New Zealand over the past decade aimed at attempting to improve our understanding of the diet of spiny lobster phyllosomes.

Key words: spiny lobster, phyllosoma, diet, feeding behaviour, mouth-parts, food chain tracing

During the 1990's scientists at the National Institute of Water and Atmospheric Research undertook research aimed at developing methods for the larval rearing of two native species of spiny lobster in New Zealand, *Jasus edwardsii* and *Sagmariasus verreauxi* (Moss *et al.*, 2001; 2004; Tong *et al.*, 1997; 2000a; 2000b; 2000c; 2000d). Small numbers of pueruli were produced for both species after extensive culture periods in 1996 *J. edwardsii* in 416 days, and in 1999 *S. verreauxi* in 241 days. The key issues for culturing these larvae over such an extensive period were keeping them in suspension in culture, maintaining adequate water quality and feeding. The suspension of these naturally pelagic larvae was achieved through the use of an upweller tank (Illingworth *et al.*, 1997), however, difficulties with maintaining water quality (Diggles *et al.*, 2000) and feeding led to research on larval culture being paused whilst research on water conditioning bacteria (probiotics) and the natural diet of phyllosoma were progressed. Spiny lobster phyllosomes are extremely difficult to study in their natural pelagic environment due to their cryptic morphology, behaviour, and their relatively low abundance in the ocean. These are important reasons why there are no recorded observations of spiny lobster feeding in the wild (Cox and Johnston, 2003b). In the absence of opportunities for direct observation, indirect research approaches need to

be used to elucidate the natural diet of phyllosomes. The research approaches used in New Zealand fell into five broad categories; feeding behaviour in captivity; feeding structures (appendages and mouthparts); digestive capacity (gut morphology and enzyme profiles); prey tracer studies (lipid biomarkers and genetic tools), and ecological associations. The results of the subsequent research on natural diet of phyllosoma have been outlined in this manuscript under these five research headings.

Feeding behaviour in captivity

Observations of the feeding behaviour of larvae in captivity is a useful technique for determining the methods for capturing and handling prey involved in feeding. This in turn can allow inferences to be made about the kind of natural prey items that might be captured in the wild, and also assist in the selection of cultured live prey, or for the design of artificial diet particles. Studies were undertaken of the feeding behaviour of the early larval development stages of *J. edwardsii* and *S. verreauxi* (Cox and Bruce, 2002; Nelson *et al.*, 2002; Cox, 2004). Unfortunately, insufficient culturing resources were available to also examine behaviour in later developmental stages. The studies mostly involved analyzing video recordings of feeding events of cultured phyllosomes that were either

Received: November 25, 2005

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free-swimming or tethered.

These studies were useful in demonstrating that the early phyllosoma stages of both species are opportunistic feeders, capable of grabbing relatively large prey items using their pereopods, usually after chance encounters with potential prey items. The captured prey was quickly and efficiently subdued and moved into position over the mouth ready for consumption. The feeding behaviour was consistent between the first three instars of both species suggesting that they do not specialize or change their diet over this period of development.

Feeding structures: appendages and mouthparts

Video recordings of the feeding of phyllosomes in captivity followed by subsequent microscopy of feeding appendages and mouthparts in a wide range of larval stages of *J. edwardsii* and *S. verreauxi* were useful in determining how the morphology of these structures related to feeding on potential prey items (Cox and Bruce, 2002; Nelson *et al.*, 2002; Cox and Johnston, 2003a; 2003b; 2004). Again, from these studies it was possible to make inferences about the types of natural prey items that could be utilized by phyllosomes in their natural environment. The examination of the pereopods of phyllosomes indicated that they had hydrodynamic and chemosensory setae which were involved in tactile and chemically induced prey capture behaviour. The mouthparts of the early larval stages were better suited to soft or fleshy prey items. However, there were marked changes in the structure of the mouthparts of *S. verreauxi* observed through the developmental stages indicating that their ability to handle and devour larger and more robust prey items increased dramatically in later larval stages.

Digestive capacity: gut morphology and enzyme profile

Video recordings of the feeding of larvae in captivity also allowed for observations of movement of food material into and through the gut. In addition, histological examination of the gut, and the identification and measurement of gut enzyme activities allowed for inferences to be made about

possible natural diets (Nelson *et al.*, 2002; Cox, 2004; Cox and Johnston, 2004; Johnston *et al.*, 2004). Video observations of feeding showed that food was sucked into the foregut through peristalsis of the oesophagus and contractions of lobes of the digestive gland. The processing of food by the gut was found to become increasingly complex with larval development and this was mirrored in the greater complexity and robustness of the digestive system. For example, the foregut in the early phyllosomes of *S. verreauxi* consists of a simple chamber with two dorsal and ventral grooves and a few very small setae on the ventral floor. In mid-stage larvae the setae are more numerous and well developed, as are the dorsal and ventral grooves of the anterior foregut, which has also become more cuticularised. The ventral chamber has also become folded in order to form a filter press. In late-stage larvae this filter press has become even more complex and efficient. Changes in the gut morphology suggest that phyllosomes probably undergo marked dietary shifts at around instars 3 and 4 and again at instars 8 and 9. The gut of early instar phyllosomes appears well suited to processing soft prey such as fish larvae or gelatinous zooplankton, while later instars should be able to process much larger, fleshier prey, such as small crustaceans. Analyses of gut enzymes of phyllosomes of *J. edwardsii* tended to support this same conclusion with analyses of both cultured and wild larvae indicating they were all capable of readily digesting dietary protein, lipid, and carbohydrate, including chitin at all stages of development. Gut enzyme activities increased significantly with larval development, reflecting an increase in digestive capacity of later larval stages. There was a marked increase in protease, trypsin and amylase enzyme activity at stages VI and VII consistent with a marked change in diet. Overall, the results indicated that the larvae are capable of digesting a wide range of prey, but they will make best use of prey that are rich in protein and lipid.

Prey tracer studies: lipid biomarkers and genetic tools

Research on the distribution and abundance of phyllosomes and pueruli of *J. edwardsii* in the

offshore waters off New Zealand and *P. cygnus* off Western Australia has led to opportunities to investigate their biochemical composition and that of by-catch species which could include potential prey species (Jeffs *et al.*, 1999; 2001a; 2001b; 2002; 2004; 2005; Phleger, *et al.*, 2001; Wells *et al.*, 2001; Bradford *et al.*, 2005; Phillips *et al.*, 2006). These studies indicate that lipid plays an important role in the energetics and development of phyllosomes. Late-stage phyllosomes accumulate large amounts of lipid (up to 34.4% of dry mass in late-stage *J. edwardsii*) material mostly in a phospholipid format which is a relatively unusual storage lipid among marine organisms. These energy stores appear to be important for fueling the subsequent non-feeding puerulus stage. The phyllosomes have well developed lipid metabolic capabilities, such as being able to convert a variety of sterols to cholesterol, alter lipid chemical structures, and to selectively utilise different fatty acids while retaining others. Therefore, attempts to use fatty acid and sterol food chain tracing methods to identify the prey of phyllosomes were not particularly insightful (Jeffs *et al.*, 2004). However, the results indicated that phyllosomes are likely to be opportunistic predators that feed on a variety of prey. Differences in the fatty acid profile between mid- and late-stage larvae were thought to be consistent with a possible dietary shift to some crustacean prey, such as shrimps, copepods and amphipods. The fatty acid food chain tracing techniques were useful in revealing the nature of the food web operating in oligotrophic conditions commonly found in the offshore waters east of New Zealand and Western Australia (Jeffs *et al.*, 2004; Phillips *et al.*, 2006). These food webs are typified by low productivity and high abundance of gelatinous zooplankton. This in itself would tend to suggest that gelatinous zooplankton would be an obvious food source for phyllosomes as they are large slow moving prey, with soft and fleshy bodies, and offering a relatively rich nutrient source. The difficulties with using fatty acid food chain tracing techniques with phyllosomes resulted in the recommendation to attempt to use emerging molecular genetic tools to identify prey species (Jeffs *et al.*, 2004). Extractions of genetic material from the hepatopancreas of mid- to late-stage phyllosoma

of the Japanese spiny lobster *Panulirus japonicus* have been recently used to identify the presence of DNA from Cnidaria and Urochordata (Suzuki *et al.*, 2006). These results provide further support to the suggestion that gelatinous zooplankton play a key role in the nutrition of spiny lobster phyllosomes

Ecological associations

Studies of the distribution and abundance of phyllosomes of *J. edwardsii* in relation to potential prey species in the offshore waters of New Zealand are also helping to provide some insight into the predatory habits of these larvae (Bradford *et al.*, 2005; Jeffs *et al.*, 2006). Analyses of the catch of phyllosoma and by-catch species from trawls with a MIDOC net indicated that the vertical and horizontal distribution of by-catch of some taxonomic groups was strongly correlated with phyllosomes which may indicate an association of phyllosoma with their prey. The abundance of phyllosomes from all developmental stages (5-11) were found to be strongly correlated with total zooplankton biomass and the biomass of gelatinous zooplankton captured in the samples. The abundance of mid-stage (5-8) phyllosomes were also strongly associated with shrimp biomass, while the abundance of late-stage (9-11) phyllosomes was strongly associated with amphipod biomass. These developmental differences were also reflected in the vertical distribution of phyllosomes with the mid-stages tending to remain in surface waters both day and night (<20 m) while the late-stages moved into deeper water during the day (20-50 m) along with the dominant potential prey category, gelatinous zooplankton. A gradual upwards shift in the vertical distribution of phyllosomes in the water column from stage 8 onwards was identified, which may also reflect a possible shift in diet of the phyllosoma. Overall, the results of this study when taken in the context of previous studies on phyllosoma feeding abilities and behaviour, strongly suggest that phyllosomes are preying on gelatinous zooplankton, as well as some small crustacean prey, and that there are marked changes in the prey between the mid- and late-stages of development.

Conclusions

Overall, the results of the various indirect studies of the natural diet of phyllosoma strongly suggest that phyllosomes are opportunistic predators that prey on a variety of species, but probably rely on gelatinous zooplankton when living in oligotrophic waters, as well as some small crustacean prey. There are marked changes in the prey between the mid- and late-stages of larval development that are evidenced through several indirect research methods. These dietary changes are likely to be associated with the late-stage larvae taking on a more active predatory role, pursuing more active prey with higher nutritive returns, such as larger crustaceans (*e.g.*, shrimps and amphipods). The application of emerging genetic “fingerprinting” techniques has the promise to further pinpoint the prey species of spiny lobster phyllosoma. The results of the studies to date would tend to suggest that artificial feeds should be soft to fleshy in texture and rich in protein and lipid. Given the size of some gelatinous zooplankton, and the body form of phyllosomes, it is possible that phyllosomes attach to living gelatinous zooplankton and feed on them for some time. This being the case, it may be possible to use static feed stations in phyllosoma culture systems.

Acknowledgements

I would like to thank all the researchers and institutions that have assisted in advancing this research into the natural diet of spiny lobster phyllosomes. Thanks also to the National Research Institute of Aquaculture, Fisheries Research Agency for their support and hosting of the International Workshop on Spiny Lobster Seed Production Technology for which this work was prepared.

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Crustacean nutrition and larval feed, with emphasis on Japanese spiny lobster, *Panulirus japonicus*

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Abstract Crustacean nutritional studies, such as requirements for proteins, amino acids, lipids, fatty acids, vitamins, minerals, etc., are reviewed, concerning with the fact that the feeding requirement of crustaceans differs between species (for example, carnivorous species require feeds with high contents of protein and lipid, compared with omnivorous and herbivorous species). This information is useful in determining the optimum dietary level of some nutrients. However, little information is available on the nutritional requirements of the spiny lobster, *Panulirus japonicus*, in particular during the phyllosoma stage.

So far, it has been established that only *Artemia* and gonads of the blue mussel, *Mytilus galloprovincialis*, are effective as foods for phyllosoma. It is very difficult to rear phyllosoma to reach to the puerulus stage with a high survival rate, even though phyllosoma are fed these kinds of feeds. It shows a lack of information on some nutritional elements in *Artemia* and mussel. The aims of our studies are to search for or develop optimal foods for the phyllosomal stage of spiny lobster *P. japonicus*. Studies on making an artificial phyllosomal feed were started with checking the size, softness, leaching, etc. of the artificial feed by rearing phyllosoma. Seasonal variations in proximate compositions of gonad of blue mussel were also monitored.

This paper provides useful information in preparing the artificial feed for phyllosoma stage of spiny lobster.

Key words: crustacean, larval feed, nutrition, spiny lobster, *Panulirus japonicus*

Crustacean nutrition

This session reviews the nutritional requirements of crustaceans and their characteristics such as requirements for protein, amino acids, lipids, fatty acids, vitamins, minerals, etc., referring to some recent reports. It has been known that the feeding requirements of crustaceans differ between species (for example, carnivorous species require feeds with high contents of protein and lipid, compared with omnivorous and herbivorous species). This information is useful in determining the optimum dietary level of some nutrients. However, little information is available on the nutritional requirement of spiny lobster *P. japonicus*, in

particular about the phyllosoma stage.

Protein requirement

Table 1 shows the published protein requirements of penaeid shrimps. To date, the protein requirements of many species have been investigated. Optimal protein levels of diets vary from 23-57%. Protein requirements of shrimps present quite large variations from one species to another. Protein requirements of *Metapenaeus macleayi* appear to be the lowest (27%), *Litopenaeus setiferus* following with 28-32%. Kuruma shrimp, *Marsupenaeus japonicus*, one of the important commercial shrimps cultured in Japan, requires 40-57% of protein in the diet. Protein level can be

Received: November 25, 2005

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as high as 55% in commercial kuruma shrimp feed. However recently we found that protein level can be reduced from 55% to 45 or 50% by the addition of probiotics in diet or in rearing water (Mochizuki and Takeuchi, unpublished data). The nutrient digestibility in shrimp, and the environmental bacterial stability as well as water conditions might be improved. Protein requirement of black tiger, *Penaeus monodon*, the most popular aquaculture species, is lower than that of kuruma shrimp. White shrimp, *Litopenaeus vannamei*, requires about 40% protein in diets. Such inter-specific variability can in part be explained by the different feeding habits of different species of shrimp, and it suggests that the protein requirements need to be studied for specific species.

Crustaceans require ten kind of essential amino acids, such as arginine, methionine, valine, threonine, isoleucine, leucine, lysine, histidine, phenylalanine, and thryptophan. These characteristics are the same as that of fish.

Carbohydrate requirement

Kuruma shrimp requires 24% maltose in diet (Kanazawa, 1995). In general, shrimps require disaccharides (such as maltose, trehalose, *etc.*) and polysaccharides (such as dextrin, starch, glycogen, *etc.*), rather than monosaccharides (such as glucose, galactose, *etc.*). In particular, more than 20% glucose supplementation may retard the growth of kuruma shrimp. Beneficial effects of di- and poly-saccharides on crustaceans are the same as that on fish, such as carp and red sea bream. It is well known that the absorption speeds of glucose derived from saccharides relates to the turnover rate of carbohydrate metabolism such as glycolysis relates to the gluconeogenesis in fish and shellfish.

Lipid requirement

Table 2 shows the published requirements for essential fatty acids (EFA) and their biosynthesis in crustaceans. Kuruma shrimp requires 6.5-16.5% lipids in the diet. EFA requirement of kuruma

Table 1. Published requirements of protein for penaeid shrimp

Species	Protein requirement (% dry matter)	Reference
<i>Metapenaeus macleayi</i>	27	Maguire and Hume, 1982
<i>M. monoceros</i>	55	Kanazawa <i>et al.</i> , 1981
<i>Marsupenaeus japonicus</i>	40-57	Balazs <i>et al.</i> , 1973 Deshimaru and Kuroki, 1975 Deshimaru and Yone, 1978 Teshima and Kanazawa, 1984
<i>Penaeus monodon</i>	35-50	Lee, 1971 Bages and Sloane, 1981 Alava and Lim, 1983 Bautista, 1986 Shiau <i>et al.</i> , 1991
<i>Farfantepenaeus aztecus</i>	40-51	Venkataramiah <i>et al.</i> , 1975 Zein-Eldin and Corliss, 1976
<i>F. californiensis</i>	35	Colvin and Brand, 1977
<i>Litopenaeus setiferus</i>	28-32	Andrews <i>et al.</i> , 1972
<i>L. merguensis</i>	50-55	Aquacop, 1978
<i>L. vannamei</i>	38-40	Smith <i>et al.</i> , 1985 Pedrazzoli <i>et al.</i> , 1998
<i>L. indicus</i>	43	Colvin, 1976
<i>L. esculentus</i>	40	Hewitt, 1992
<i>L. plebejus</i>	47	Aquacop and Cuzon, 1992
<i>L. stylirostris</i>	35	Colvin and Brand, 1977

(Allan and Smith, 1988; partially modified)

Table 2. Published requirements and biosynthesis of essential fatty acids for crustaceans

Species	EFA requirement and Biosynthesis	Reference
Post-settlement		
<i>Marsupenaeus japonicus</i>	1% (DHA=EPA>LNA>LA)	Kanazawa <i>et al.</i> , 1979
<i>Penaeus chinensis</i>	(DHA>AA>LNA>LA)	Xu <i>et al.</i> , 1994
<i>P. monodon</i>	1% DHA (LNA $\nrightarrow$ EPA $\nrightarrow$ DHA)	Merican and Smith, 1996
<i>Farfantepenaeus aztecus</i>	1% LNA	Shewbart and Mies, 1973
<i>Macrobrachium borellii</i>	LNA $\nrightarrow$ EPA; 20:3n-6 $\nrightarrow$ AA	Gonzalez-Baro <i>et al.</i> , 1998
<i>Panulirus japonicus</i>	EPA $\nrightarrow$ DHA	Kanazawa and Koshio, 1994
Larvae		
<i>Scylla serrata</i>	0.8% n-3HUFA (rotifer feeding) 0.7-0.9% EPA and 0.5-0.7% DHA LNA $\nrightarrow$ EPA; LA $\nrightarrow$ AA (<i>Artemia</i> feeding)	Suprayudi <i>et al.</i> , 2002 Suprayudi <i>et al.</i> , 2004
<i>S. paramamosain</i>	1.3-2.5% EPA and 0.5% DHA (<i>Artemia</i> feeding)	Kobayashi <i>et al.</i> , 2000
<i>Portunus tribuberculatus</i>	0.9-1.7% HUFA (rotifer feeding)	Takeuchi <i>et al.</i> , 1999
Phyllosoma		
<i>Panulirus cygnus</i>	Long-chain PUFA (<i>Artemia</i> feeding)	Liddy <i>et al.</i> , 2005
<i>Jasus edwardsii</i>	Need high percentage of DHA (<i>Artemia</i> feeding)	Nelson <i>et al.</i> , 2004
Broodstock		
<i>Macrobrachium rosenbergii</i>	1.3% LA and 1.5% n-3 HUFA	Cavalli <i>et al.</i> , 1999

shrimp juvenile is 1% docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA), but a higher requirement is needed for linolenic acid (LNA). *P. monodon* also requires 1% DHA. On the other hand, *Farfantepenaeus aztecus* requires 1% LNA. As for fatty acid metabolism, larval kuruma shrimp were found to have a greater ability than juvenile to convert LNA into n-3 highly unsaturated fatty acids (n-3HUFA), such as EPA and DHA. In other shrimps, *Penaeus chinensis* cannot convert LNA into EPA and EPA into DHA, while *P. monodon* can convert EPA into DHA. Larval crabs, such as the mangrove crabs, *Scylla serrata* and *S. paramamosain*, require n-3HUFA. They need higher levels of n-3HUFA at *Artemia* feeding stages than that at rotifer feeding stages, and also they cannot convert LNA into EPA and linoleic acid (LA) into arachidonic acid (AA). Examined via radioactive tracer experiments, Kanazawa and Koshio (1994) pointed out that spiny lobsters may lack the capacity for the bioconversion of EPA and DHA into other fatty acids. So, it can be

considered that DHA and/or EPA are essential fatty acids for spiny lobster. In particular, phyllosoma of *Panulirus cygnus* and *Jasus edwardsii* need high levels of n-3HUFA or DHA during the *Artemia* feeding stages.

Phospholipids are also essential for crustaceans. Phosphatidylcholine (PC) and phosphatidylinositol (PI) are required, while phosphatidylethanolamine (PE) and phosphatidylserine (PS) have no nutritional effect. Most crustaceans require 0.5-1% of phospholipids in diet, except for freshwater prawn, *Macrobrachium rosenbergii* (Coutteau *et al.*, 1997).

Cholesterol requirement in crustaceans is about 1% in larvae and 0.5% in juveniles. Shrimps are deficient in biosynthetic capability of sterol. But C₂₈ and C₂₉-cholesterol can be converted into C₂₇-cholesterol *in vivo* (Kanazawa and Teshima, 1971; Teshima, 1998).

β -Carotene is an important nutrient for crustaceans. The usual pigments isolated from crustaceans are astaxanthin, β -carotene, echinenone

and canthaxanthin. β -Carotene is converted into astaxanthin via cryptoxanthin, echinenone or canthaxanthin, respectively (Linan-Cabello and Paniagua-Michel, 1998). On the other hand, the pathway of bioconversion of the main dietary carotenoids in shrimps and *Artemia* is producing retinoic acid. It is well known that retinoic acid is one of the elements incurring abnormality. Overdose of vitamin A and retinoic acid causes bone deformity in fish. However, it has never been investigated in studies on lobsters. β -carotene is also a precursor of vitamin A. Addition of vitamin A in feeds could improve the growth of larval shrimps and be essential for normal development of the ovaries. The recommended supplementation level of vitamin A in shrimp feed is about 1500 IU/100 g dry weight of diet (Linan-Cabello *et al.*, 2002), that is nearly the same as fish.

Vitamin and mineral requirements

Nutritional requirements of vitamins and minerals are similar to fish (Kanazawa, 1995; Shiau, 1998; Pedrazzoli *et al.*, 1998). Recommended supplementation level of ascorbic acid (vitamin C) is 1% in the diet. This value is 100 times higher than that of dietary requirement. It is mainly on account of the behavior of shrimp feeding. Before shrimp ingest the feed, nutritional elements, especially water soluble vitamins, are leached. Therefore a high level supplementation of vitamin C is required.

More interesting results of the studies on vitamin C requirement of crustaceans showed that kuruma shrimp and spiny lobster lack biosynthetic capacity of vitamin C, while American lobster has. Nearly the same results are obtained in studies on fish. Rainbow trout do not have the ability of biosynthesis of vitamin C, while carp have. Thus, it is necessary to check the biosynthetic capacity of vitamin C in each species of crustacean, and to examine the supplementation levels of the water soluble nutrients in the diet.

Calcium (Ca) and phosphorus (P) ratio is also important for crustaceans. Kuruma shrimp and American lobster require 1:1 and 1:2 of Ca:P, respectively (Kanazawa, 1995). It is well known that most crustaceans and fish can absorb Ca directly from seawater.

To date, the nutritional studies on crustaceans have focused on the investigation of shrimps. Little information has been reported concerning about nutritional requirements of lobsters, especially about phyllosoma and juveniles of spiny lobster *P. japonicus*. Further research is needed to clarify the nutritional requirement of phyllosoma.

Seed production and quality of foods in spiny lobster *Panulirus japonicus*

Seed production of spiny lobster has been conducted and puerulus and first stage juvenile

Table 3. Puerulus and juvenile production in Minamiizu Station

Year	Phyllosoma 1.5mm Times of Molting	Phyllosoma Duration	Puerulus 3cm	Juvenile
1990	30 (29-31)	292.2 (280-304)	2	2
1991	28.4 (25~30)	286.4 (266-306)	5	4
1992	29.0 (20~31)	317.5 (231-417)	67	21
1993	27	293	1	0
1994	26.6 (26-27)	308.8 (234-393)	144	54
1995	-	280.7 (256-314)	6	2
1996	-	312.0 (237-385)	59	31
1997	-	318.2 (256-385)	41	30
1998	-	373.0 (253-517)	113	49
1999	-	360.5 (321-405)	14	5
2000	-	338.3 (281-408)	17	7
2001	-	338.7 (316-370)	3	1
2002	-	328.6 (240-426)	120	78
2003	-	360.3 (288-537)	51	39
In total	20-31	231-537	643	323

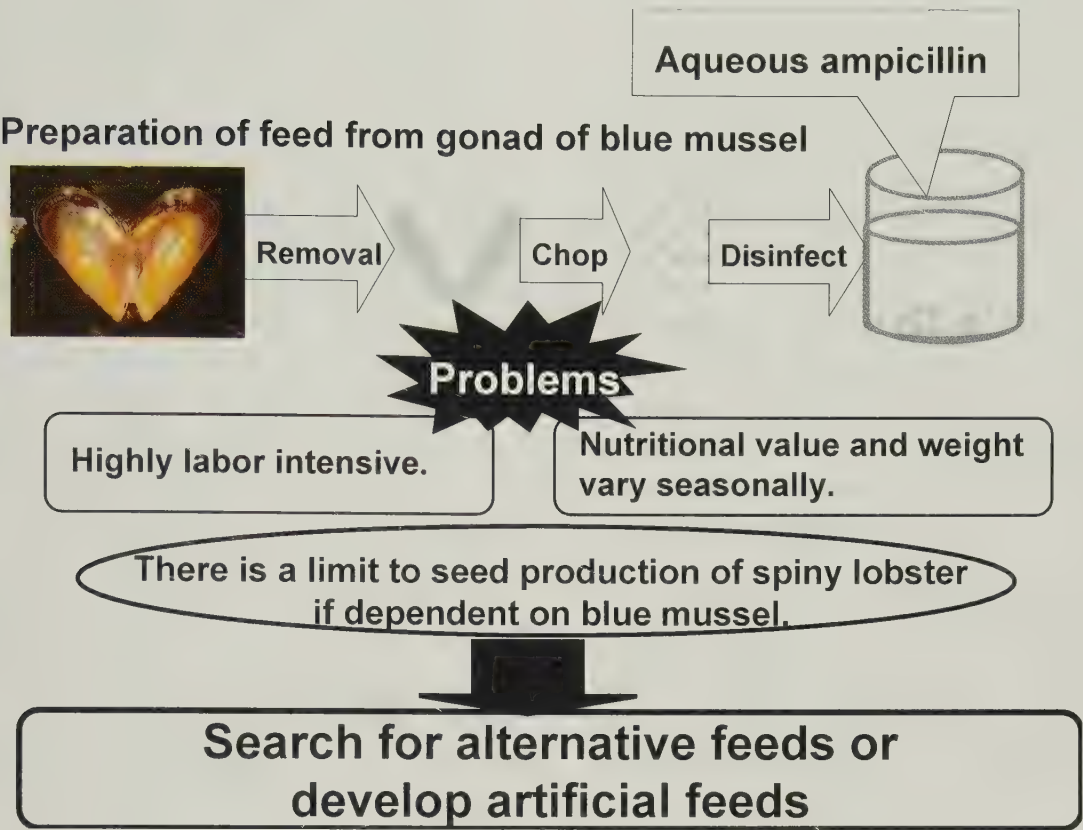


Fig. 1. Process of the preparation of feed for phyllosoma

have been obtained since 1990 (Table 3). Duration of phyllosoma is nearly 300 days, and molting times are about 30. Then phyllosoma metamorphose into puerulus. Only about 50% of puerulus can be successfully metamorphose to the juvenile stage. During the approximate 14-day puerulus stage, the larvae do not feed. So the quality of phyllosoma feed is very important for obtaining first lobster juvenile. So far, it has been known that only *Artemia* and gonads of blue mussel *Mytilus galloprovincialis* are effective as phyllosoma feeds. It is very difficult to make phyllosoma attain to puerulus at a high survival rate, even though phyllosoma were fed these kinds of feeds. It shows a lack of information on some nutritional elements in *Artemia* and mussel gonads. The aims of our studies are to search for or develop optimal foods for phyllosomal stage of spiny lobster *P. japonicus*. Studies in making an artificial phyllosomal feed were started with checking the

size, softness, leaching, *etc.* of the artificial feed by reared phyllosoma. Seasonal variations in proximate compositions of gonads of blue mussel were also monitored.

Problems of blue mussel feeding

The preparation process for phyllosomal feed has met some problems (Fig. 1). The preparation process includes several steps: first, the shells of live blue mussels have to be opened; and then the gonads removed from the shells; after that the gonads are chopped into small pieces with a knife; finally in order to prevent infection, the minced gonads are immersed in 20 ppm ampicillin solution over night.

These processes is not only labor intensive, but also the nutritional values of the live mussel gonads of each lot needs to be checked because of their seasonal variation. Mass seed production depending on blue mussel gonads will be extremely difficult.

So it is necessary to search for alternative foods and to develop artificial feed. If phyllosoma could be fed on alternative foods, these foods will be easy to obtain in large quantities throughout the year and easier to prepare than blue mussel gonad. If phyllosoma could be fed on artificial feed, it will be easy to adjust the dietary size to changes in the phyllosomal size, easy to regulate the nutrient composition, and save labor.

Rearing conditions are also important for successful rearing of phyllosoma. So far, it is very difficult to make mass production of spiny lobster, because phyllosoma are very fragile. For example, when several individual phyllosoma are reared together in a small vessel, the long legs of individual phyllosoma interweaved each other and easy to be broken, and survival of phyllosoma is easy to be influenced by deterioration of the water condition due to uneaten food, etc. Recently, a new type of vessel - rotary vessel, was developed. This rotary vessel was shown to be effective to decrease the intertwine of individuals and increase the chance of meeting with feeds. In seed production, survival rate of larvae from phyllosoma to juvenile increased from 10% to 30% at the maximum. Also it is very

important to introduce the hygienic treatment of rearing water, such as ultraviolet radiation, disinfectant, *etc.* Now rearing methods are being improved.

Seasonal variation of blue mussel

In seed production, phyllosoma are fed with *Artemia* enriched with *Phaeodactylum* during phyllosomal stages I to V. Then, phyllosoma are fed with gonad of blue mussel together with *Artemia*, but mainly with gonad of blue mussel. After the spawning season, the volume of mussel gonad becomes low. Since ovary is more effective compared with testis, usually testis and small size gonad are not used as phyllosoma feed.

Year-round monitoring and comparison of nutritional compositions were conducted. Harvested blue mussels were divided into two groups (samples 1 and 2, S1 and S2) by visual check. Usually, only S1 were used in seed-production. Difference in ratio of ovary weight/shell length was found between S1 and S2 (Fig. 2). In all seasons, values of S1 are higher than that of S2. The ratio decreased during the spawning period, especially in S1. Fig. 2 also shows the seasonal variation of crude ash, glycogen

Table 4. Nutritional levels of blue mussel *Mytilus edulis* during a year-round

Protein	30-70%	(Min.: Apr.)
Glycogen	0.5-40%	(Min.: Jan.)
N-3HUFA	1.5-3.9 g/100g	(Min.: Apr.)
Amino acids		(Min.: Dec.)

Table 5. Proposal for the development of artificial feeds for phyllosoma during the period of spiny lobster seed production project

Year	Research details
2005-2006	Analyzing effective compositions of blue mussel, alternative and natural foods
2005-2007	Gaining a clear understanding of feed shape for chewing and screen for alternative feeds
2006-2008	Developing new artificial feeds to achieve the process of metamorphosis and molting

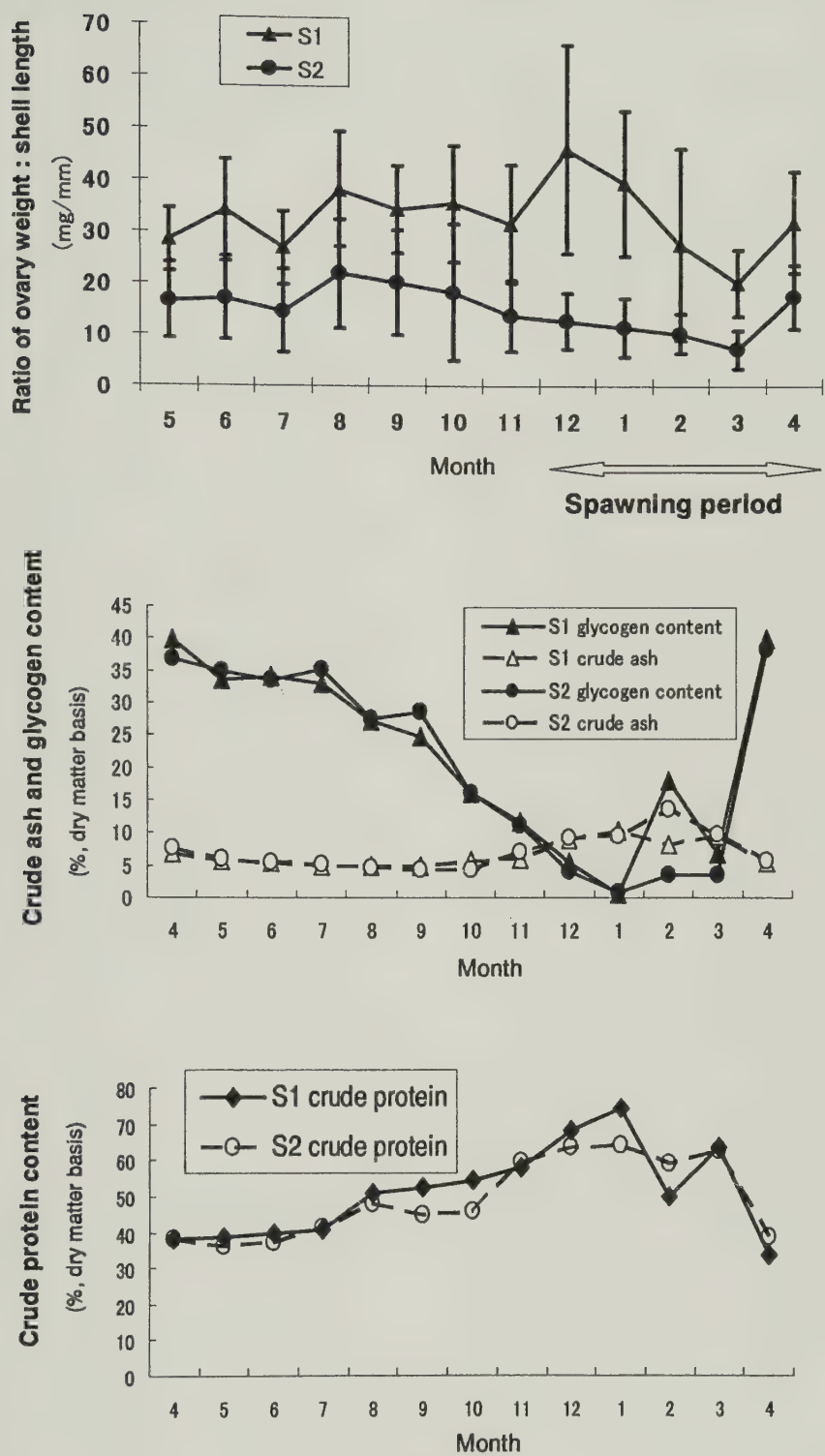


Fig. 2. Seasonal variation in ratio of ovary weight / shell crude (in upper figure), ash and glycogen content (in middle figure), and crude protein content (in lower figure)

content, and crude protein. Glycogen content in both S1 and S2 groups reached the maximum of 40% in April whereas the minimum of 0.5% in January. Crude protein content in both S1 and S2 groups reached the maximum of 70% in January whereas the minimum of 30% in April. The seasonal variation between crude protein and glycogen contents showed the contrary results. N-3HUFA in total lipids in both S1 and S2 groups reached the maximum of 3.9 g/100 g in January whereas the minimum of 1.5 g/100 g in April (Table 4). These patterns are the same as crude protein content (Fig. 2). Polar lipid contents in gonad mussel also showed the same pattern. On the other hand, amino acid contents reached the minimum in the spawning period. This result is the same as glycogen content. So all the nutritional contents have seasonal variations and showed wide ranges. We recommend that the best season for blue mussels to feed to phyllosoma is autumn, just before spawning, considering the nutritional balance of each element. The each value of blue mussel in this season is as follows; ratio of ovary weight/shell length, 30-35 mg/mm; crude protein content, 55-60%; glycogen content, 10-15%; polar lipids, 4.5-5.0%; nonpolar lipids, 7-8%; and n-3HUFA, 3.0 g/100 g.

Conclusion

From this year, we start a project of semi-mass production of spiny lobster. The subject is the development of artificial feeds for phyllosoma (Table 5). Nutritional compositions of blue mussel, alternative and natural foods are planned to be analyzed in 2005 and 2006. For example, seasonal variations of proximate compositions, amino acids compositions, lipid classes and fatty acid compositions of blue mussel have been investigated and the best season of high mussel quality has been found, as mentioned above. Some other kinds of natural foods will also be analyzed.

We aim to have a clear understanding of feed shape for chewing, and select ideal alternative feeds before the end of 2007. Our final goal is to develop a new artificial feed to achieve the process of metamorphoses and molting not later than 2008.

Acknowledgements

We would like to thank Dr. Lu Jun of Tokyo University of Marine Science and Technology, for her useful suggestions and assistance.

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Feeds development for post-larval spiny lobster: A review

Kevin C. WILLIAMS *

Abstract On-growing of spiny lobster from wild seed is a thriving aquaculture industry in SE Asia and most notably in Vietnam where production of the tropical lobster *Panulirus ornatus* is estimated at more than 2,000 tonnes per annum. Present culture relies on lobsters being fed fresh fishery by-catch. The dwindling supply of these feeds and their downstream environmental impact have increased the priority to develop sustainable and more eco-friendly pelleted feeds. Efforts to develop palatable and high performance pelleted dry feeds for spiny lobster grow-out is reviewed in this paper with particular emphasis on Australian research with temperate (*Jasus* spp.) and tropical (*P. ornatus*) species. This research has increased our understanding of the lobster's requirements for critical nutrients such as protein and total lipid and progressed the development of pelleted dry feeds for lobster grow-out.

Key words: rock lobster, nutrition, digestibility, protein, attractants

Spiny lobsters (Palinuridae) are one of the world's most valuable seafoods with high market appeal in Asia, Europe and America. Most capture lobster fisheries are either over-exploited and in decline or are being managed for their maximum sustainable yield (Phillips, 2000, 2005). Aquaculture appears to be the only long-term way of meeting market demand for spiny lobsters. Although laboratory-scale rearing of the larvae from egg to puerulus has been achieved for many species, including *P. elephas*, *P. japonicus*, *P. longipes*, *Jasus lalandii*, *J. edwardsii* and *J. verreauxi* (Kittaka, 2000; Matsuda and Yamakawa, 2000), commercially-viable hatchery production of spiny lobsters is still thought to be a long way off. Until successful hatchery technology is developed, the only practical way of increasing the volume of marketed lobster is to capture juveniles from the wild and on-grow them to market size, thereby circumventing the high natural mortality that otherwise would occur (Phillips *et al.*, 2003). The tropical ornate lobster *Panulirus ornatus* is probably the best candidate for aquaculture as it has the shortest oceanic larval development phase of 4-6 months (Dennis *et al.*, 2001) and the fastest

post-larval growth rate, capable of attaining a market size of 100-105 mm carapace length (~1 kg) within 18 months after settlement (Phillips *et al.*, 1992; Skewes *et al.*, 1997; Dennis *et al.*, 1997; Hambrey *et al.*, 2001).

The on-growing of *P. ornatus* is a flourishing industry in many parts of SE Asia and notably in Vietnam where the abundance of wild lobster juveniles has enabled lobster grow-out aquaculture to develop rapidly with 2,000 tonnes, worth US\$70-75 M, being harvested in 2001/02 (Thuy and Ngoc, 2004). In Vietnam, lobsters are fed exclusively on fresh fishery by-catch. However, the diminishing supply and increasing cost of by-catch, together with the downstream environmental impacts of this type of feeding, are strong incentives for the development of more eco-friendly pelleted lobster feeds.

Despite more than 30 years of research to develop a suitable compounded (artificial) feed for rearing juvenile spiny lobsters (see Conklin, 1980; Booth and Kittaka, 1994; Brown *et al.*, 1995), progress has been slow and our knowledge of their nutritional requirements is still sparse. However, progress in developing suitable pelleted dry feeds for spiny

lobster grow-out has gained considerable momentum in the past 5-10 years. This paper reviews this more recent research and identifies areas where further research is critically needed.

First principles of feeds development

Knowing what the animal prefers to eat in the wild is a useful guide when developing an artificial feed for the animal. Analysis of the contents of the foregut of postlarval spiny lobsters reveals a wide variety of molluscs (predominantly, bivalves gastropods and chitons), crustaceans (predominantly barnacles, crabs and other decapods), polychaete worms, echinoderms and occasional (incidental?) amounts of macroalgae (Joll and Phillips, 1986; Booth and Kittaka, 1994; Barkai *et al.*, 1996; Mayfield *et al.*, 2000; Goni *et al.*, 2001). This diet selection characterizes them as opportunistic carnivores of predominantly benthic invertebrates. Thus they most likely have evolved to most efficiently utilize foods that are high in protein, low in lipid and moderate to high in starch since glycogen is the major energy store of molluscs and typically 14-24% of the ash-free dry matter (Dall *et al.*, 1991; Lodeiros *et al.*, 2001; Orban *et al.*, 2002). Studies on the digestive enzymes of juvenile and adult spiny lobsters attest to their carnivorous feeding preference with high proteolytic (trypsin, chymotrypsin and carboxypeptidase A), moderate carbohydrase (α -amylase, β -glucosaminidase, laminarinase and cellobiase) and comparatively low lipase activities (Barkai *et al.*, 1996; Johnston, 2003; Radford *et al.*, 2005). Interestingly, amylase and laminarinase specific activities were reported to decrease as a function of lobster size, implying that carbohydrate might be a more important dietary constituent for juveniles than adult spiny lobsters (Johnston, 2003).

An understanding of what attracts an animal to its food is also helpful when developing an artificial diet. A lot is known about chemoreception in marine decapods (see Lee and Myers, 1997; Grasso and Basil, 2002), and feeding preferences of spiny lobsters are also becoming better understood (Derby, 2000; Derby *et al.*, 2001; Grasso and Basil, 2002). Like homarid lobsters, spiny lobsters

have a well developed antennular chemosensory system for locating food, finding shelter and social interactions with other lobsters. Characteristically, feed attractants for crustaceans are low molecular weight, water and ethanol soluble, and amphoteric or basic compounds that are likely to be released from potential prey items. Thus to ensure an artificial feed will be perceived by the lobster as suitable food, it should leach a steady plume of attractants rich in free amino acids, especially taurine, glycine, arginine, glutamic acid and alanine, and other low molecular weight organic compounds such as organic acids, nucleotides and nucleosides, betaine or small peptides (Lee and Myers, 1997).

Since shrimp and lobsters show similar behavioural responses to chemical cues (Daniel *et al.*, 2001), it is not surprising that commercial shrimp pellets have been tested to see if lobsters would eat and grow well on them. In Australian work with juvenile *P. ornatus*, *P. cygnus* and *J. edwardsii*, formulated shrimp pelleted feeds were readily eaten by the lobsters but their growth and survival were generally much poorer than those fed mussel flesh (Crear *et al.*, 2000, 2002; Glencross *et al.*, 2001; Smith *et al.*, 2003). These observations suggest that the shrimp pellets were either nutritionally inadequate for, or not sufficiently attractive to, the lobsters. The latter appeared to be the most likely explanation as lobsters would cease feeding on the shrimp pellets within a 1-2 h of being offered whereas the attractiveness of mussel flesh persisted for 10 or more h.

Development of pelleted dry feed

General approach

The method used to develop an artificial feed for any newly husbanded animal typically follows one of two pathways: an iterative process based on diluting the animal's natural food with increasing amounts of less-natural constituents or a more structured process whereby the animal's requirements for key nutrients are defined and the ability of alternative feed ingredients to supply these nutrients is determined. Sometimes these two approaches may become blurred, especially in the early stages of artificial feed development when natural food

items are often used to improve the acceptance of compounded diets being used for nutritional requirement studies. This certainly has been the case with spiny lobsters where mussel flesh or other prey items have commonly been included in formulations to improve the acceptance of the pelleted feed (Crear *et al.*, 2000, 2002; Glencross *et al.*, 2001; Smith *et al.*, 2003; Perera *et al.*, 2005). This approach has enabled the nutritive value of potential feed ingredients to be determined and the lobster’s requirements for critical nutrients to be further defined.

Nutritive value of feed ingredients

In considering the usefulness of a feed ingredient as a source of nutrients for assimilation by the animal, the likelihood of growth inhibitors or

contaminating toxins being present must be considered. A thorough critique of this aspect is beyond the scope of this review. For an account of naturally occurring growth inhibitors and toxic contaminants, readers are referred to reviews by Hardy (1999), Francis *et al.* (2001), Hendricks (2002) and Burgos-Hernandez *et al.* (2005). Poor storage or handling of the ingredient can also diminish its value since bacterial contamination and/or oxidative decomposition during or after production can destroy critical nutrients or produce toxins such as biogenic amines (Aksnes and Mundheim, 1997; Opstvedt *et al.*, 2000; Shakila *et al.*, 2003). However, from a pure nutritional perspective, nutritive value of an ingredient is typically first assessed by measuring its apparent digestibility. Sadly, very little information is available on the apparent

Table 1. Apparent digestibility (mean ± SE) of ingredients of potential use in artificial grow-out feeds for spiny lobsters

Ingredient	Dry matter	Crude protein	Total lipid	Gross energy	Ref
Apparent digestibility coefficient (%)					
<i>Marine protein sources</i>					
NZ blue mussel meal		97.6 ±0.1			1
NZ green-lipped mussel meal	76.4 ±3.0	88.8 ±1.98	63.7 ±6.04	83.3 ±2.45	2
Shrimp head meal		77.2 ±19.1			1
Crustacean meal (Lango)	53.2 ±3.0	85.2 1.98	53.4 ±6.04	72.0 ±2.45	2
Fish meal (67% CP)		62.5 ±1.4			1
Fish meal (67% CP)	67.4 ±3.0	84.2 1.98	78.1 ±6.04	80.7 ±2.45	2
Squid meal		7.3 ±2.3			1
Krill meal	68.5 ±3.0	88.6 ±1.98	64.4 ±6.04	77.5 ±2.45	2
<i>Terrestrial protein sources</i>					
Lupin flour		100.1 ±6.0			1
Wheat gluten		90.1 ±9.7			1
Soybean meal		60.5 ±19.0			1
Pea meal		52.0 ±8.7			1
Canola meal		38.3 ±13.7			1

¹Measured in *J. edwardsii* (Ward *et al.*, 2003a)
²Measured in *P. ornatus* (Unpublished data: S.J. Irvin and K.C. Williams, CSIRO Marine & Atmospheric Research)

digestibility of feed ingredients for spiny lobster and this is an area where further work is needed. Table 1 provides data on the apparent digestibility of some feed ingredients that have been used in feeds development research for spiny lobsters. As for penaeid shrimp (Smith *et al.*, 2001) and mud crab (Catacutan *et al.*, 2003), spiny lobsters digest the protein of animal and some plant meals with high efficiency. Particularly interesting is the high apparent digestibility of lupin and wheat gluten protein, a finding that is similar to that observed for fish and other crustaceans (Smith *et al.*, 2001). However, the apparent digestibility of the protein in commercial squid meal measured in *J. edwardsii* was unexpectedly low (7 %) and suggests that excessive heating and protein denaturation during the manufacturing process may have been the cause. Whether or not that result is typical of processed squid meal or an aberrant batch of the product is unknown. Clearly, more work needs to be done to measure the digestibility of feed ingredients that have potential to be used in artificial feeds for spiny lobsters. With *P. ornatus*, difficulties in collecting a representative sample of faeces have been overcome by using a novel balloon method, which prevents voided faeces coming in contact with the water (Irvin and Tabrett, 2005).

State of nutritional knowledge pre-2000

Several reviews on the nutritional requirements of spiny lobsters have been published (Conklin, 1980; Kanazawa, 1994). However, the paucity of work with spiny lobsters meant that information gathered on the clawed homarid lobsters was heavily relied upon for these reviews. Kanazawa and Koshio (1994) and Teshima (1997) compared the essential lipid nutrition of the Japanese spiny lobster *P. japonicus* with that of homarid lobster and penaeid shrimp and concluded that essential fatty acid, phospholipid and sterol requirements of these crustaceans are all very similar. However, it must be stated that less than a handful of studies were relied upon in reviewing the essential lipid requirements of spiny lobsters. The intent of this paper is not to present a comprehensive review of spiny lobster nutrition but rather to update our knowledge on this subject by examining more recent research and how this

is being used to develop high performance pelleted feeds for grow-out of spiny lobsters.

State of protein and lipid knowledge post-2000

A coordinated Australian growth study examined the dietary protein and total lipid requirements of very small (<4 g initial weight) *P. cygnus*, *P. ornatus* and *J. edwardsii* lobsters (Glencross *et al.*, 2001; Smith *et al.*, 2003; Ward *et al.*, 2003). Six pelleted dry feeds containing incremented amounts of crude protein (from 35 to 60% dry matter) at each of two levels of total lipid (6 and 10%, dry matter) were prepared centrally and distributed to the collaborating laboratories. An additional diet of either fresh blue mussel, *Mytilus edulis* or a kuruma (*Penaeus japonicus*) shrimp feed was included in the experimental array as a reference. In each of the experiments, lobster survival was good (>75%) and growth improved curvilinearly with increasing dietary protein content but the response depended on both the lipid content of the feed and the species of lobster. With *P. cygnus*, the best growth occurred with feeds containing 55% crude protein (DM) and was better for the low- than the high-lipid feeds (Glencross *et al.*, 2001). With *P. ornatus*, the best growth occurred at calculated dietary protein asymptotes of 47 and 53% (DM) for the 6 and 10% lipid feeds, respectively; at the higher protein levels, lobster growth was better for the high- compared to the low-lipid feeds (Smith *et al.*, 2003). With *J. edwardsii*, dietary lipid level had no significant effect on the response to protein with growth being best at calculated dietary digestible protein asymptotes of 33-35%, dry matter (equivalent to 42-47% crude protein, dry matter) (Ward *et al.*, 2003b). Eight pelleted feeds containing serially incremented protein (from 35 to 60%, dry matter) at a constant total lipid content of 9% dry matter were examined in another study with larger *J. edwardsii* (initial weight of ~ 70 g) (Crear *et al.*, 2001). As with small lobsters, growth rate of the larger lobsters improved curvilinearly with increasing dietary protein but the response flattened out only when the dry matter crude protein content of the feed was greater than 50%. In all four studies, the reference feed (mussel flesh or kuruma shrimp pellets) resulted in significantly better growth, often

twice as good, as the best experimental feed. These results suggest that different spiny lobster species have different dietary protein and lipid requirements and thus may require feeds specifically tailored for each species. However, as the growth of the lobsters on the pelleted experimental feeds was poor, it is questionable whether these results truly were a reliable measure of the animals' dietary protein and lipid requirements

Improving the acceptance of pelleted dry feeds

A common observation in all of the above studies was that lobsters would readily eat the pelleted experimental feed when first given but would then be ignored after being immersed in the water for 1-2 hours. This contrasted with the response to mussel flesh where lobsters continued to feed after it was in the water for 10 or more hours. This observation prompted experiments to examine why the attractiveness of pelleted feed diminished after such a short time of immersion. Working with juvenile *J. edwardsii*, Tolomei *et al.* (2003) measured the excitatory capacity and the attractability of shrimp pellets and mussel flesh after these had been immersed in water for periods of up to 8 h. They also examined the chemoattraction of different concentrations of glycine, taurine and betaine. The lobster's attraction to shrimp pellets and mussel flesh declined with increasing immersion time. However, feeding shrimp pellets that had been soaked for periods of 0.5, 2, 4 or 8 h did not affect the growth, feed conversion or survival of the lobsters during a 12-week growth trial. The greatest feeding behavioural response (antennule flicking) of the lobsters occurred with glycine at concentrations of 10^{-4} to 10^{-6} mol/L while the concentration of taurine had to be increased to 10^{-2} mol/L to get a similar high behavioural response; the response to betaine remained low at all concentrations over the range 10^{-8} to 10^{-2} mol/L.

A quite different response was observed with juvenile *P. ornatus* (Williams *et al.*, 2005). They sought to characterize feeding cues by quantitating the nitrogenous compounds leaching from mussel flesh or pelleted dry feeds following immersion in water over a period of 7.5 h and correlating the leachate with the preference of the lobsters to the

same soaked or non-soaked feeds. Homogenates of natural prey items, either polychaete, shrimp, mussel or squid, were included in the pelleted feed so that the chemical signatures of the leachates would be different. The lobster's feeding preference was most positively correlated with the amount of soluble protein, glycine and taurine that leached from the feeds. However, lobsters showed a much higher preference for mussel flesh than for the pelleted dry feeds even when 5-h soaked mussel was compared with non-soaked pelleted feed. These results suggested that increasing feeding frequency and including protein hydrolysates and other free amino acid-rich ingredients in the dietary formulation might be practical ways for prolonging the attractiveness of pelleted artificial feeds. Floreto *et al.* (2001) used krill hydrolysate to enhance the acceptance of soybean-based feeds for the American lobster, *Homarus americanus*, and found that soybean could provide almost 90% of the dietary protein with no adverse effects on growth relative to feeding mussels.

Re-examination of protein requirements

To test the value of including krill meals in feed formulations for spiny lobsters, an experiment was carried out with juvenile *P. ornatus* to re-examine dietary protein requirements (Smith *et al.*, 2005). Freeze-dried krill hydrolysate and freeze dried krill meal were used at a constant inclusion rate of 8 and 30%, respectively, and the protein content of the feed serially increased by adding incremental amounts of fish meal at the expense of starch to produce five isolipidic feeds that varied from 34 to 61% crude protein dry matter (31 to 56% digestible crude protein dry matter). An additional diet of thawed green-lipped mussel, *Perna canaliculus*, was included in the treatment array as a reference. The formulation and determined chemical composition of these feeds are detailed in Table 2. During the 8-week experiment, growth rate of the lobsters fed the pelleted feed four-times daily continued to increase with increasing dietary crude protein content, with no suggestion of the response plateauing even at the highest level examined (61%, dry matter; 56% digestible, dry matter) (Fig. 1). Thus, juvenile *P. ornatus*, require high dietary

Table 2. Formulation and key chemical composition* of feeds used in re-evaluating the dietary protein requirement of juvenile *P. ornatus* (Smith *et al.*, 2005)

Attribute	Feed label					
	33CP	40CP	47CP	54CP	61CP	Mussel
<i>Formulation (% as used)</i>						
Fish meal	0	9.0	18.0	27.0	36.0	
Starch	27.1	20.4	13.6	6.8	0	
Fish oil	4.4	3.3	2.2	1.1	0	
Diatomaceous earth	5.6	4.4	3.3	2.2	1.1	
Wheat flour	14.0	14.0	14.0	14.0	14.0	
Krill meal	30.0	30.0	30.0	30.0	30.0	
Krill hydrolysate	8.0	8.0	8.0	8.0	8.0	
Other ¹	10.9	10.9	10.9	10.9	10.9	
<i>Composition (dry matter basis)</i>						
Crude protein	33.8	40.4	46.6	53.6	61.2	57.4
Dig. CP	31.0	36.8	41.9	48.9	56.3	nd
Lipid	13.0	13.7	13.8	12.8	12.8	11.5
Gross energy (kJ/g)	19.6	19.6	20.2	20.5	20.8	19.6
Digest. energy (kJ/g)	17.9	18.2	18.4	18.7	19.0	nd

*See Smith *et al.* (2005) for more information on the other ingredients and the chemical composition of the feeds

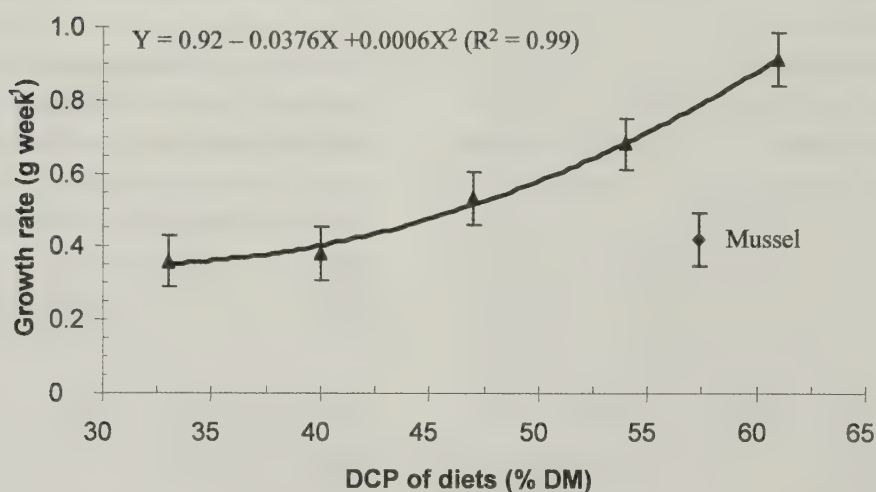


Fig. 1. Relationship between the digestible crude protein (DCP) content of the feed (on a dry matter basis) (X) and growth rate of *P. ornatus* lobsters fed pelleted feeds for the 0-8 week period of the growth response experiment (Y). The growth rate of lobsters fed thawed green-lip mussel is shown at its corresponding crude protein content (dry matter basis). Error bars are $\pm$ SEM.

protein specifications (viz, >60% crude protein, dry matter; >56% digestible crude protein, dry matter) to achieve high growth rates on pelleted feeds.

Adequacy of mussel flesh as sole feed

Interestingly, lobsters fed the thawed mussel in the experiment of Smith *et al.* (2005) grew well for the first four weeks but thereafter the growth rate of the lobsters progressively declined and deaths, mainly at times of moulting, increased. Survival of lobsters fed thawed mussel was only 41% compared to 73-84% for lobsters fed pelleted feeds. Moreover, mussel-fed lobsters became increasingly pale in colour, and those that died were invariably very pale or pink-tinged. Junio-Menez and Ruinata (1996) also found thawed green mussel, *Perna viridis*, to be an inadequate sole feed for juvenile *P. ornatus* with only 6% of lobsters surviving the 4-month experiment. In a New Zealand study with juvenile *J. edwardsii*, fresh blue (*Mytilus galloprovincialis*) or green-lipped (*P. canaliculus*) mussel resulted in significantly better growth and survival than feeding 3-day aged mussels or frozen mussel (James and Tong, 1997). These studies collectively indicate that freezing may have deleteriously altered the nutritional quality of the mussel, perhaps reducing vitamin potency or altering the bioavailability of some critical micronutrient. The paleness of the lobsters fed the mussel in the study of Smith *et al.* (2005) suggested a sub-optimal intake of astaxanthin.

Dietary carotenoid and astaxanthin

Carotenoids are an expensive (about US\$3,000/kg of active astaxanthin) and critical component of crustacean feeds but little is known of the carotenoid requirements of tropical spiny lobsters. Most animals are unable to synthesise carotenoids *de novo* and thus are dependent on an exogenous dietary supply to meet their requirements (Meyers and Latscha, 1997). In crustacean and fish, astaxanthin is the predominant carotenoid. It is stored as free astaxanthin or as astaxanthin esters where the astaxanthin molecule is attached to a fatty acid. Astaxanthin has many functions in crustaceans. It has been demonstrated to be important in reproductive performance (Pangantihon-Kuhlmann *et al.*, 1998; Pérez-Velazquez *et al.*, 2003) and in

larval and post-larval development (Petit *et al.*, 1997; Pan *et al.*, 2001). It also appears to have an important role as an antioxidant and to be involved in immunocompetence and stress tolerance (Meyers and Latscha 1997; Linan-Cabello *et al.*, 2002; Chien *et al.*, 2003). In crustaceans, astaxanthin is not readily synthesised from other ingested carotenoids, with β -carotene having the highest bio-conversion efficiency of about 50% (Meyers and Latscha 1997).

Crear *et al.* (2002) evaluated six commercial shrimp pelleted feeds and fresh blue mussel, *M. edulis* as feeds for juvenile *J. edwardsii*. Three of the shrimp feeds were formulated for the kuruma shrimp, *P. japonicus* and the other three for the black tiger shrimp, *Penaeus monodon*. These feeds were fed to slight excess for 134 days and measurements made of growth performance, carapace colour and body composition of the lobsters. Lobsters grew significantly better on the mussel than on the shrimp pelleted feeds but other productivity traits did not differ greatly between the various feeds. However at the conclusion of the experiment, carapace colour of the lobsters differed markedly between the feeds, with colour scores being markedly higher for the three kuruma shrimp feeds (4.2-5), lowest for the black tiger shrimp feeds (1.4-1.8) and intermediate for the mussel (4). Highly significant curvilinear and linear relationships were found between dietary carotenoid content and the lobster's carapace colour and tissue carotenoid content, respectively. These authors concluded that lobsters need a dietary carotenoid level of around 115 mg/kg to ensure a carapace colouration score of >4, equivalent to that of wild-caught juvenile *J. edwardsii*.

The poor growth, low survival and pale exoskeleton colouration of juvenile *P. ornatus* fed frozen green-lipped mussel in the work of Smith *et al.* (2005) prompted a further study to examine the dietary astaxanthin requirement of this species (Barclay *et al.*, 2006). They carried out a 12-week experiment with juvenile *P. ornatus* fed either pelleted feeds supplemented with astaxanthin (providing total dietary carotenoid contents of 30, 60, 90 or 120 mg/kg) or one of two frozen mussel reference feeds-blue, *M. edulis*, or green-lipped, *P. canaliculus*, mussels. The pelleted feeds were based

on the formulation of the best feed in the earlier study (Feed 61CP; Table 2) except that astaxanthin was incrementally added to produce the desired dietary astaxanthin specifications. Neither growth rate nor survival showed a dose response to dietary astaxanthin but lobsters fed the two mussel feeds consistently grew more slowly and survival tended

to be lower, especially during the last 4-weeks of the experiment (Fig. 2A). Exoskeleton colour increased directly as the dietary carotenoid content of the pelleted feeds increased but the colour of lobsters fed the mussel was only poorly related to the carotenoid content of the mussel (Fig. 2B). Similarly, whole body total carotenoid content of the

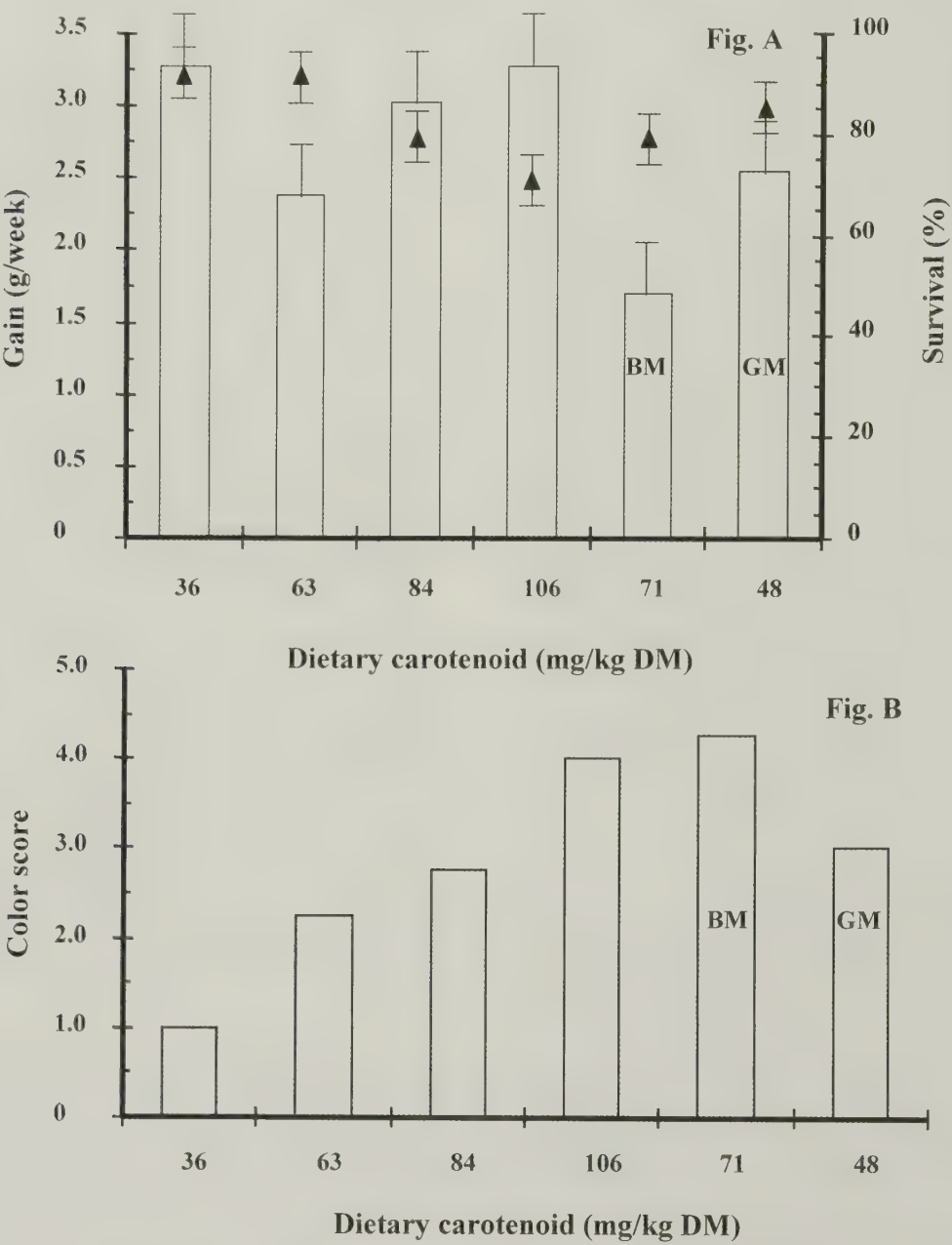


Fig. 2. Effects of dietary carotenoid on growth (bars) and survival (▲) (Fig. A) and on exoskeleton color score (Fig. B) of juvenile *P. ornatus* fed either pelleted feeds providing incremental supplements of free astaxanthin or frozen blue, *M. edulis* (BM) or frozen green-lipped, *P. canaliculus*, (GM) mussel. Error bars are $\pm$ SEM ($n = 4$).

lobsters increased linearly with increasing dietary carotenoid for the pelleted feeds but the relationship was less clear for the mussel-fed lobsters (Fig. 3A). However, most of the carotenoid in the mussel was not astaxanthin, but other pigments most likely originating from the microalgae that had been consumed. When whole body astaxanthin content of the lobsters was examined in relation to the

free astaxanthin content of the feed, an excellent curvilinear relationship was seen for all feeds (Fig. 3B). Although the range of dietary astaxanthin examined in the experiment did not affect lobster productivity, it did markedly affect exoskeleton colour and tissue astaxanthin content, which could have important implications on the animal's immunocompetence and on the market acceptance of

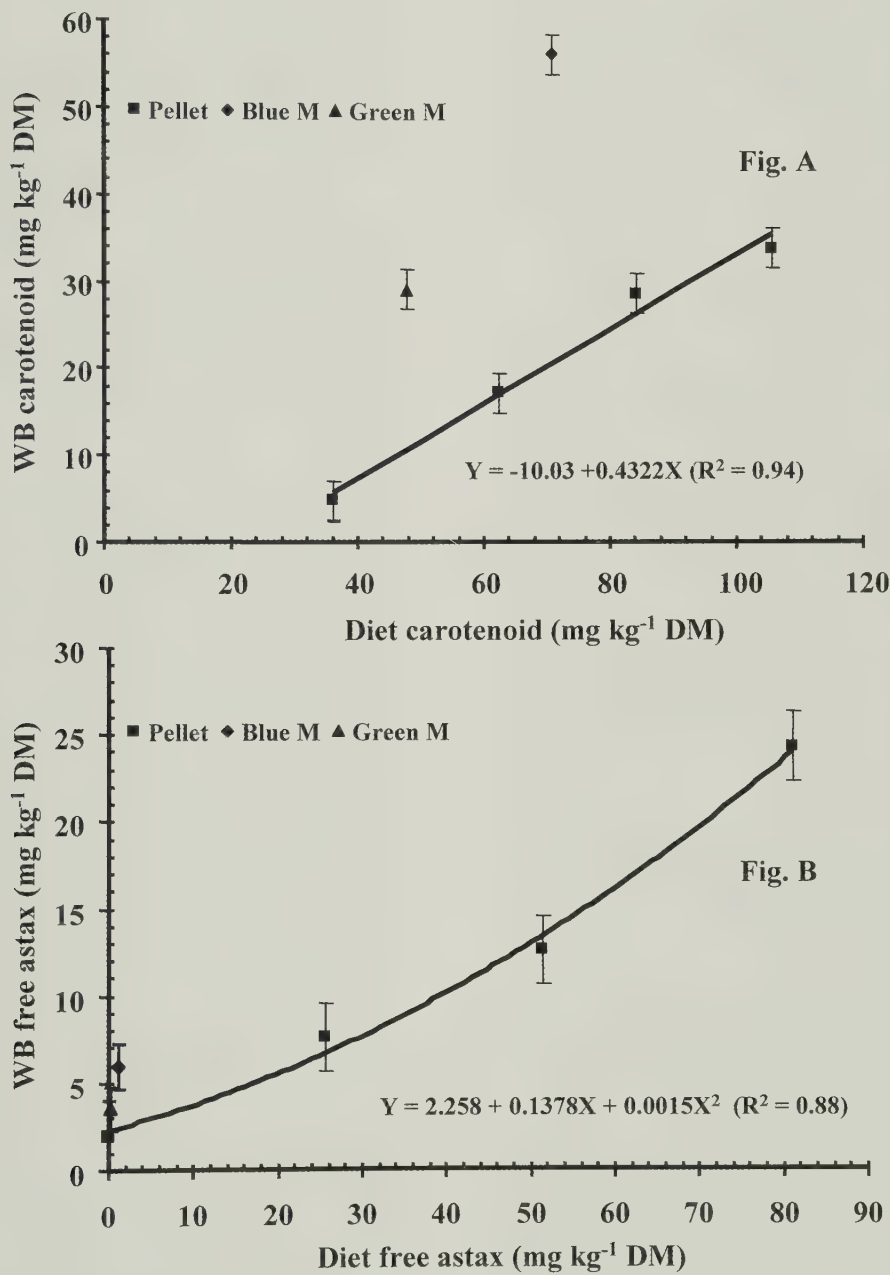


Fig. 3. Effects of dietary carotenoid content and whole body (WB) carotenoid content (Fig. A) and dietary free astaxanthin (astax) content and free astax content (Fig. B). Error bars are $\pm$ SEM (n = 4).

the cultured lobster. Moreover, the study confirmed the findings of the earlier study (Smith *et al.*, 2005) that frozen mussels are not a suitable sole feed for *P. ornatus*.

Neither the study of Crear *et al.* (2002) nor that of Barclay *et al.* (2006) were able to demonstrate an improved productivity response to dietary carotenoid, and thus could not define a true requirement. However, both studies showed dietary carotenoid supply had a clear and marked effect on lobster colouration, a feature that has considerable impact on the marketing and the price paid for the lobster. If for this reason alone, a total dietary carotenoid specification of at least 100 mg/kg, and an astaxanthin equivalent of not less than 70 mg/kg, is recommended.

Future research perspectives

Much progress has been made in the past five years or so to better define the nutritional requirements of post-larval spiny lobsters and to develop suitable compounded pelleted dry feeds. This research has shown that the tropical *P. ornatus* grows best when provided with feeds that are high in digestible protein (>56 % dry matter) and with 10-11 % total lipid dry matter. More temperate species such as *P. cygnus* and *J. edwardsii* appear not to need as high a dietary protein or lipid specification as *P. ornatus* but this conclusion needs further validation. Very little recent research has been done to quantify the lobster's requirements for essential lipids, especially sterol, phospholipid and essential fatty acids, and this will assume greater importance when terrestrial feed ingredients are used to replace marine sources in cheaper dietary formulations. A compounded artificial feed that is well accepted by *Panulirus* and *Jasus* lobsters and which produces good growth and survival has been developed. However, this formulation presently requires high inclusion rates of krill products, which are expensive. The task ahead for researchers will be to develop less expensive feed formulations that lobsters readily accept and grow well on. This will require more information on the apparent digestibility and acceptability of alternative and cheaper feed ingredients, most likely of terrestrial

rather than marine origin, and further definition of the lobster's requirements for critically important nutrients and energy.

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Mass mortality and its control in the larval rearing of brachyuran crabs: Implications for mass culture techniques of phyllosoma larvae

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Tadao JINBO^{*3} and Shigeki DAN^{*4}

Abstract Larval mass mortality has been reported during the large scale seed production of brachyuran crabs in Japan, but rearing larvae of brachyuran crabs is relatively easy in small vessels. In this paper, we discuss the gaps between rearing methods of larval snow crabs using large tanks and small vessels, and show the results of a preliminary study to control the mass mortality of snow crab larvae in large tanks. Based on these results, we identify the direction for research on the mass culture of phyllosoma larvae of the Japanese spiny lobster *Panurilus japonicus*.

Key words: Brachyuran crabs, seed production, larval rearing, mass mortality, behaviour, phyllosoma

The Fisheries Agency of the Japanese Government initiated marine stock enhancement programmes in 1963 to augment and stabilize fishery resources. Brachyuran crabs were targeted in the stock enhancement programmes and we studied the seed production technologies of brachyuran crabs. These crabs included: horse hair crabs *Erimacrus isenbekii*, which are distributed in northern parts of Japan, especially in Hokkaido; snow crabs *Chionoecetes opilio*, which inhabit cold waters in the deep sea; swimming crabs *Portunus trituberculatus*, and mud crabs *Scylla serrata* and *S. paramamosain*, which inhabit warm waters (Hamasaki, 2002, 2003; Hamasaki *et al.*, 2002a, b; Arai *et al.*, 2004; Kogane *et al.*, 2005; Jinbo *et al.*, 2005). In the seed production of these species, newly hatched larvae are stocked in large tanks with flow-through water systems. Rotifers and *Artemia* sp. are daily supplied, at designated densities, to the tanks as food for the larvae. Phytoplankton are also added to the tanks as food for rotifers and *Artemia*.

Studies on the artificial rearing of larvae examined the ecology and physiology in the early life history

of many brachyuran species all over the world (see Anger, 2001). In these studies the general method of larval rearing is as follows: newly hatched larvae are stocked in small vessels with seawater and prey, such as rotifers and *Artemia*. Larvae are daily transferred into new vessels with new seawater and prey using a large mouthed pipette.

We have experienced larval mass mortality during the seed production of brachyuran crabs, but rearing larvae of brachyuran crabs is relatively easy in small vessels. For example, in our rearing experiments, the survival rate of snow crab larvae in 500 L tanks rapidly decreased and few larvae moulted to the second zoea (Kogane and Hamasaki, unpublished data); however, in one litre beakers, mean survival rate to the first crab stage reached *ca.* 30 % (Kogane *et al.*, 2005) (Fig. 1). Differences between survival rates in large tanks and small vessels were also observed for other brachyuran species (*e.g.*, Hamasaki, 2003; Hamasaki *et al.*, 2002a).

Why does mass mortality occur in large tanks? In this paper, we discuss the gaps between rearing methods of larval snow crabs using large tanks and

Received: November 25, 2005

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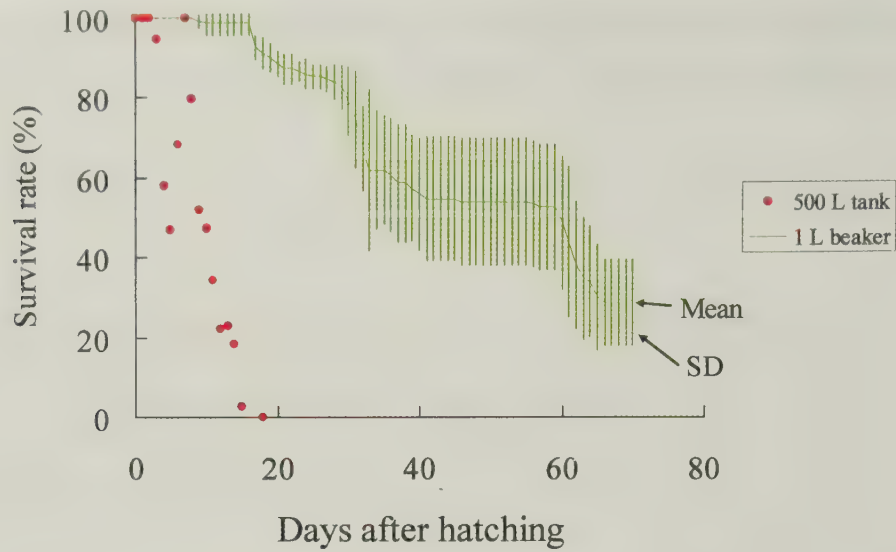


Fig. 1. Survival rates of snow crab larvae reared in a 500 L tank and 1 L beakers. Unpublished data from Kogane and Hamasaki are shown for a 500 L tank. Survival rates of larvae reared in five 1 L beakers at 14°C are shown (Kogane *et al.*, 2005).

small vessels and show the results of a preliminary study to control the larval mass mortality in large tanks. Based on these results, we identify the direction for research on mass culture of phyllosoma larvae of the Japanese spiny lobster *Panurilus japonicus*.

Gaps between large tanks and small vessels

During larval rearing in tanks, organic matter such as dead larvae, prey, and faecal pellets gradually accumulate on the tank bottom. Based on our observations, snow crab larvae are distributed in the upper water for a while after hatching. Then, they gradually lose their planktonic habit and sink to form patches on the tank bottom. Patches may be formed by water currents in the tanks. Larval snow crabs have long dorsal and ventral spines. We can assume that these spines are easily broken when the crabs are in patches on the tank bottom. Pathogenic bacteria increase due to decaying organic matter and infect injured larvae, causing mass mortality.

On the other hand, in small vessels, mass mortality does not occur because we can keep the rearing environment almost constant by transferring the larvae into new vessels with new seawater and prey and remove the dead larvae. In order to control

the larval mass mortality in large tanks, we should prevent the larvae from sinking to the tank bottom and control bacterial disease.

Larval behavioural characteristics

To prevent the larvae from sinking to the tank bottom by controlling larval behaviour, we examined the phototaxis and geotaxis of larval snow crabs (Kogane and Hamasaki, unpublished data). Phototaxis was examined as follows. Five light-adapted larvae were put in the centre of a horizontal rectangular chamber (100 cm long, 10 cm wide and 10 cm deep). The length of the chamber was marked at 5 cm intervals. Each larval position was checked after five minutes under light and dark conditions. Light (500 lx) was turned on from the end of the chamber. This procedure was repeated three times. Geotaxis was examined by a similar method to that used in the phototaxis experiments. Five light-adapted larvae were placed into the top of a vertical rectangular chamber (45 cm long, 10 cm wide, and 10 cm deep) with marks at 5 cm intervals. Each larval position was checked after five minutes under light and dark conditions. Light (500 lx) was turned on from the top of the chamber.

In the phototaxis experiments, larvae did not

move from the section where they were placed under dark conditions. Under light conditions, newly hatched larvae moved to the light source, i.e., showing strong positive phototaxis. Then, phototaxis gradually became weak with growth and the megalopa did not move under light conditions. In the geotaxis experiments, under both light and dark conditions, newly hatched larvae were positioned in the upper sections, i.e., showing negative geotaxis. Then, geotaxis became positive when late first zoea, second zoea and megalopa sank to the bottom. Thus, snow crab larvae sink according to their growth stages and it is difficult to prevent larvae from sinking to the tank bottom by controlling their behaviour.

Effects of water agitation and bath treatment with drugs on larval survival of the snow crabs in large tanks

To control the possible causes of mass mortality of larval snow crabs in large tanks, i.e., larval sinking and bacterial disease, we examined the effects of water agitation and treatment of tank water with drugs on survival of larvae (Kogane and Hamasaki, unpublished data).

Two rearing trials were conducted using 500 L tanks. In the first trial, newly hatched larvae were stocked in two 500 L tanks, one equipped with an agitator (Fig. 2) and the other without an agitator. The agitator rotated once per minute. The tanks

were provided with a flow-through water system (turnover rate: once/day) and aeration. Water temperature was regulated with a heating system at 14°C. Larvae were fed with rotifers and *Artemia*. In the second trial, we used the same 500 L tanks with and without the agitator used in the first trial and added sodium nifurstyrenate to both tanks at a concentration of 2 mg/L weekly.

In the first trial, all of the larvae in the tank without the agitator died within 20 days after hatching. In the tank with the agitator, the survival rates were higher than the tank without the agitator (Table 1). In the second trial, the survival rates were higher than the first trial, and those in the

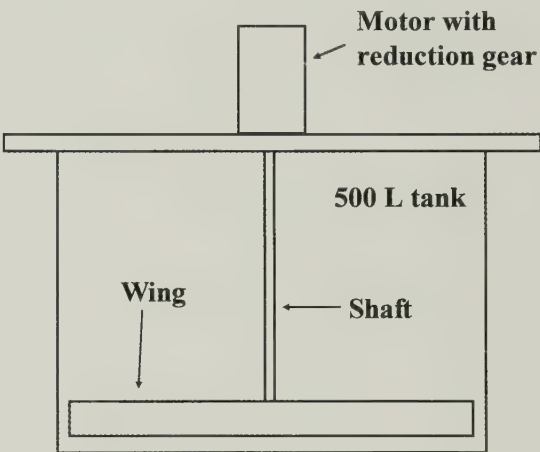


Fig. 2. Lateral schematic illustration of the agitator used for rearing experiments of the larval snow crabs in 500 L tanks.

Table 1. Survival rates of snow crab larvae reared in 500 L tanks

Expt.	Tank	Survival rate (%)			
		10 DPH	20 DPH	30 DPH	39 DPH
I	Agitator	53.3	11.3	2.5	-
	Control	47.3	0.0	-	-
II	Agitator	72.7	63.6	25.5	22.3
	Control	42.7	52.7	13.6	11.6

DPH: days post hatching

Bath treatment with sodium nifurstyrenate (2 mg/L) was conducted once every week in Expt. II

tank with the agitator were higher than the tank without the agitator (Table 1). In both trials, rates of swimming larvae were higher in the tank with the agitator than the tank without the agitator (data are not shown).

Thus, the survival rates were improved using the agitator and sodium nifurstyrenate. Since 2002 we have applied this rearing system to larger tanks of 20 kL and shown the effectiveness of the agitator and tank water treatment with sodium nifurstyrenate. Larval survival rate was improved in the mass seed production tanks and several thousand larvae survived to the first crab stage (Fig. 3).

We improved the survival rate of snow crabs using the agitator and drugs but survival rates in larger tanks were still lower than in small vessels (Fig. 1 and Table 1). Some larvae sank to the tank bottom after the second zoeal stage even when using the agitator. In future studies, to prevent the larvae sinking to the bottom and to improve survival rates, we should study the water currents in the tanks, larval behaviour and bacterial disease in greater detail. Further, use of drugs might not be recommended for long because it can result in the selection of drug-resistant pathogenic strains. Hence, we should develop alternative measures to control bacterial diseases, *e.g.*, biological control, which is

being studied by our research group for mud crabs (Hamasaki, 2003).

Research direction for mass culture of phyllosoma larvae

The problems encountered in seed production of brachyuran crabs have also existed in the rearing of phyllosoma larvae. Their larval development period is quite long, *e.g.* >300 days (Sekine *et al.*, 2000), and they have long pereopods that are easily injured and damaged by interactions between individuals and by physical stress. Periodical tank antibiotic treatments and change of rearing vessels are essential to rear phyllosoma larvae to the juvenile stage (Murakami, personal communication), restricting the culture scale of phyllosoma to tanks about 100 L in volume.

To improve the survival rate and expand the scale of culture of phyllosoma larvae, firstly, behavioural characteristics such as phototaxis and geotaxis, and sinking speed; then, control measures to regulate larval behaviour such as using an agitator similar to that which improved the larval survival rate in the snow crabs, should be investigated. Further, it is important to study bacterial diseases and their control measures.

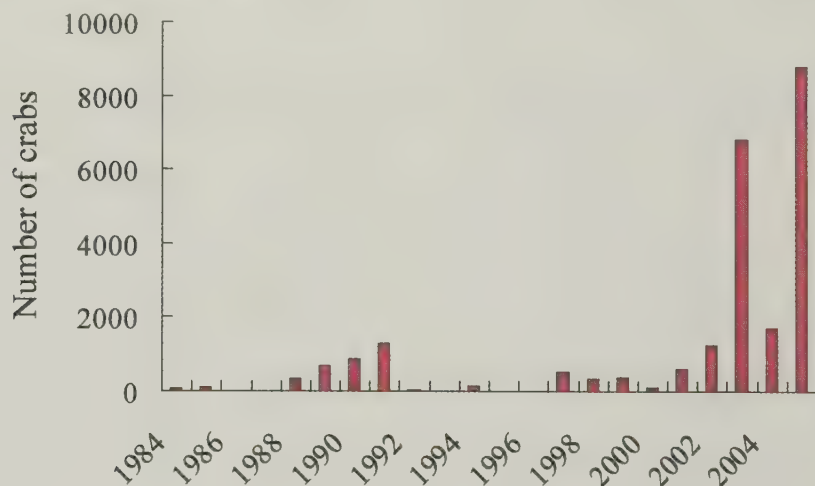


Fig. 3. Number of snow crab juveniles (first-stage crabs) produced at Obama Station of the National Centre for Stock Enhancement, Fisheries Research Agency.

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Prospects for aquaculture of bay lobsters (*Thenus* spp.)

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Abstract The Australian Fresh Research and Development Corporation Pty Ltd (AFR & DC) in association with the Department of Primary Industries and Fisheries, Bribie Island Aquaculture Research Centre (DPIF, BIARC) has been researching and developing the aquaculture potential of the Bay Lobster, *Thenus* spp. since 1995. The project was undertaken with the view of developing an economically sustainable supply of consistently high quality seafood products in controlled environments unaffected by the unpredictable forces of nature.

The AFR & DC achieved a world first by successfully rearing larvae of this species through to the juvenile stage and then to maturity. The attributes of this species, including rapid growth to commercially acceptable sizes within 13 months from hatching, ability to tolerate crowding and high market prices make it a highly attractive commercial aquaculture proposition. The AFR & DC has been conducting research to confirm these attributes in the laboratory initially and subsequently in pilot scale production. The successful pilot scale production is based on a better understanding of animal biology, including the digestive system, nutritional requirements and physical requirements.

Key words: *Thenus*, aquaculture, digestive system, commercialization

Introduction

Bay lobsters (*Thenus* spp.), locally known as the Morton Bay bugs, are members of the scyllarid lobster family and are found along the entire northern coast of Australia from Shark Bay in Western Australia to Coffs Harbour in northern New South Wales (Kailola *et al.*, 1993). There are two *Thenus* species: *Thenus indicus* (the Mud bug) and *Thenus orientalis* (The Sand bug) (Fig. 1). Mud bugs are brown overall and have brown stripes on their walking legs, while Sand bugs are speckled overall and have spots on their walking legs. Mud bugs prefer a bottom of fine mud, and are typically trawled from inshore coastal waters of 10 to 30 meters depth. Sand bugs tend to prefer sediments with a larger, coarser particle size, and are usually trawled from a depth of 30 to 60 meters in the coastal shelf and offshore areas (Courtney *et al.*, 2001). The Queensland catch of Bay Lobster

is currently 400-650 tonnes as a by-product of prawn and scallop trawling with a wharfside value of at least \$5.2-8.2 millions (Courtney *et al.*, 2001). The current minimum legal size for all *Thenus* species in Queensland is 75mm carapace width, and the capture of egg-berried females is prohibited (Courtney, 2002).

Currently, commercial aquaculture of any palinurid and scyllarid lobster species is not being carried out anywhere in the world. The major unsolved problem for developing aquaculture techniques of lobster species lies in the successful maintenance of the planktonic larval (phyllosoma) stages, where the phyllosoma stages are longer-lived (generally 150 to 300 days) and pelagic in natural habitat. Although significant efforts for advancing rearing methods of rock lobster phyllosomas have been made in recent years, mass-culture of rock lobster phyllosomas is still not feasible at this stage. However successful results have been reported from a recent study of

Received: November 25, 2005

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Fig. 1. (a) Sand bug *Thenus orientalis*, indicating spots on their walking legs and (b) Mud bug *Thenus indicus* indicating stripes on walking legs.

Thenus species, where phyllosomas of *Thenus* pass through only 25 to 30 days with high survival rate (>80 %), and juveniles can grow to market size (250 g) within 400 days in the growout phase (Mikami, 1995). This paper examines the potential to apply information and techniques obtained from rearing of *Thenus* from the experimental scale, and describes the proposed recirculation commercial scale facility for *Thenus*.

Biology of *Thenus*

Thenus spp. live on the sandy or muddy sea floor in tropical-subtropical coastal waters. Within the genus *Thenus*, *T. orientalis* is the larger of the two species and generally caught in greater depths, ranging from 30-60 m. The smaller species, *T. indicus* is generally taken in depths to about 30 m (Courtney, 2002). Females spawn as many as 60,000 eggs per individual during the summer. Two spawnings per one female during one season is common, showing two spawning

peaks in early summer (August-September) and late summer (January-February) (Kailora *et al.*, 1993). Phyllosomas hatched from eggs go through 4 moult stages, then metamorphose to the benthic nisto stage. The average total body length of newly hatched phyllosomas is 3.86 mm for *T. orientalis* and 3.67 mm for *T. indicus*, and that of final (4th) instar phyllosomas is 18.22 mm for *T. orientalis* and 16.44 mm for *T. indicus*, respectively (Mikami and Greenwood, 1997). The average duration of hatchery reared phyllosomas of *Thenus* at the temperature of 26-27°C is 25-30 days (21 days minimum), believed to be the shortest duration of the phyllosoma stage within any palinurid or scyllarid species (Mikami, 1995). After 19 moults, most juveniles can reach a typical market size of about 250 g. The time taken from eggs to market size depends on temperature and food supply, but generally ranges for 400 to 450 days. Some females can spawn eggs 1 year from hatching, but most females spawn 2-3 years after hatching (Kailora *et al.*, 1993).

Functional morphology of the lobster digestive system

There is no doubt that diet is an important factor affecting survival of phyllosomas and juveniles/adults of lobster species, and one of the key issues for successful development of lobster aquaculture. Observation of the functional morphology of the digestive system can indicate the suitability of particular food types and contribute to better understanding of the requirements for larval and growout diet developments. Knowledge derived from the study of the digestive system can also be used as an indication of whether developed diets are properly utilized by phyllosomas and juveniles/adults. There are many publications on the functional aspects of digestive system of lobster species, with review of Mikami and Takashima (2000).

The basic structure of the phyllosoma mouthparts in palinurid and scyllarid lobster species is similar; the large labrum and paragnaths form a well-developed semi-enclosed chamber covering the mandibles' top. The symmetrical coronal surface

of the mandible is divided into three portions: the canine-like process, the molar process and well-developed incisor process. It is suggested that only soft food masses can enter the semi-enclosed chamber surrounded by the labrum and paragnaths, where only "chewing" of food mass occurs by the well-developed incisor process of mandibles. The stomach of phyllosomas consists of a simple straight tube, different from that of juveniles/adults, which consists of two chambers, the cardio and pyloric chambers. The absence of a two chamber system in the phyllosoma stomach indicates that the function of the stomach is mainly squeezing and filtration of the food particles previously cut and chewed within the mouthparts (Mikami *et al.*, 1994). The midgut gland of the phyllosoma is the most noticeable structure of the digestive system with a single layer of midgut lumens occupying most of the inner carapace. The lumen of the midgut gland consists of the four different cell types (R-cells, B-cells and F-cells); and R-cells and B-cells are thought to be involved intercellular digestion (Mikami *et al.*, 1994). The R-cells are the

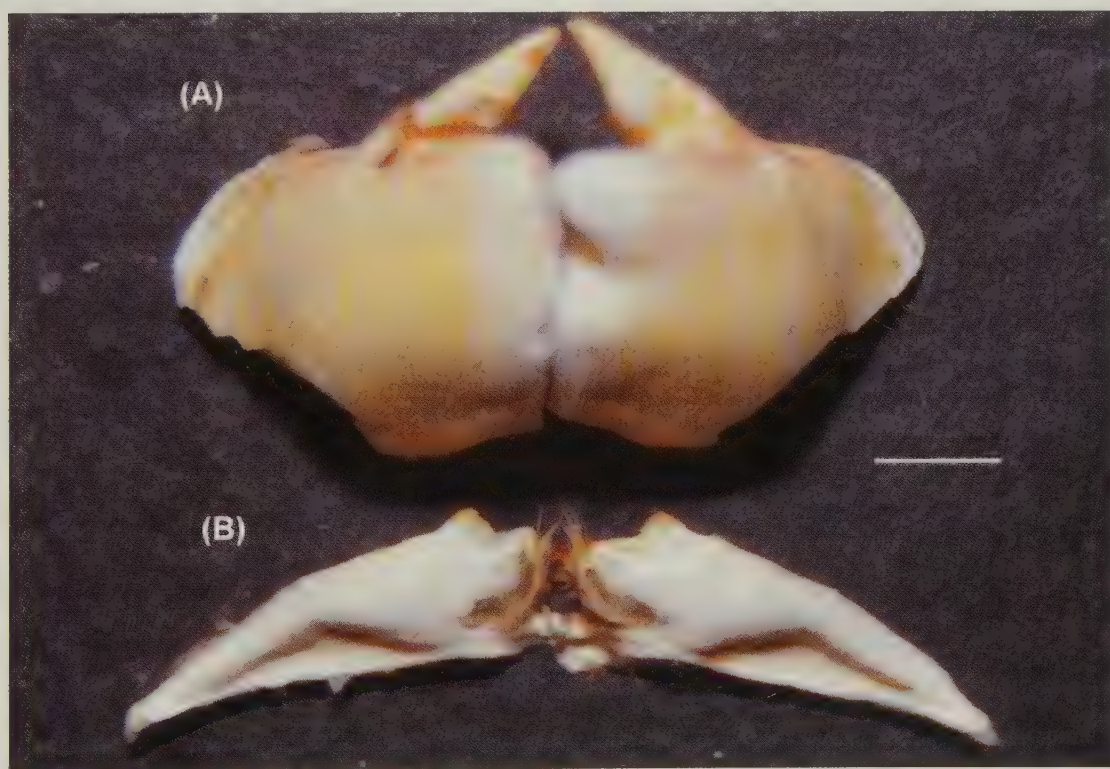


Fig. 2. The mandibles of same size adult of (A) *Panulirus longipes* and (B) *Thenus orientalis*, Scale bar represents 1cm.

most abundant cell type within the midgut gland and characteristically contain a large number of spherical globules after feeding. The R-cells absorb low molecule size digested nutrients including amino acids, carbohydrates and phospholipids by selective transportation through the apical cell membrane. The B-cells pinocytotically absorb nutrients of large molecular size from the lumen of the midgut gland and store these in vacuoles. The theory is that the B-cells store nutrients within the vacuoles, later holocrine to the lumen of the midgut gland and then they are re-digested by the R-cells. The spherical globules of phyllosoma midgut disappear within a short time (0.5-1 hour) after feeding, and also B-cells are not dominant, suggesting continuous feeding of phyllosomas for their maximum growth.

The structure of mouthparts in juvenile/adult lobsters is very different depending on species in relation to their habitat. For example, the mandibles of *Panulirus* are well developed with strong molar and canine processes, suitable for crunching hard material, like the gastropod shell. On the other hand, mandibles of *Thenus* are poorly developed, indicating they are able to chew only soft-bodied material (Fig. 2). The structure of the digestive system of juveniles/adults lobsters suggests juveniles/adults can store nutrition better than the larval stage. The stomach of juveniles/adults consists of 2 chambers (cardiac and pyloric chambers) with series of calcified ossicles, which are capable of grinding stored food into small particles. The lumens

of the midgut gland extend three dimensionally, some nutrients such as glycogens and lipids can be stored among the gaps between lumens. These adult digestive system structures suggest they are adequate to starvation and less frequent feeding for their optimal growth.

Development of larval rearing system

Three key issues have now been identified for the successful rearing of oceanic phyllosomas; 1) nutrition, 2) hygiene (bacteria/virus control) and 3) hydrodynamics (tank design). Although *Artemia* and mussel gonad have been used a food source, poor survival and growth rate records indicate that they may not be ideal in terms of quantity and quality, particularly for late stage phyllosomas. Zooplankton (*e.g.*, *Sagittas*, Copepod), hydromedusa and fish larvae have also been tested previously, but have not been successful due to difficulties in maintaining a continuous supply. The development of artificial diets is one solution for overcoming the nutritional problem.

Bacterial/viral control is another key issue for the rearing of long-lived oceanic phyllosomas under controlled environments. Traditionally, a number of antibiotics (*e.g.*, OTC, Streptomycin, Chloramphenicol) and chemicals (*e.g.*, formalin) have been used for controlling bacterial colonies in the rearing water of phyllosomas. Though antibiotics and chemicals are strong agents for minimising

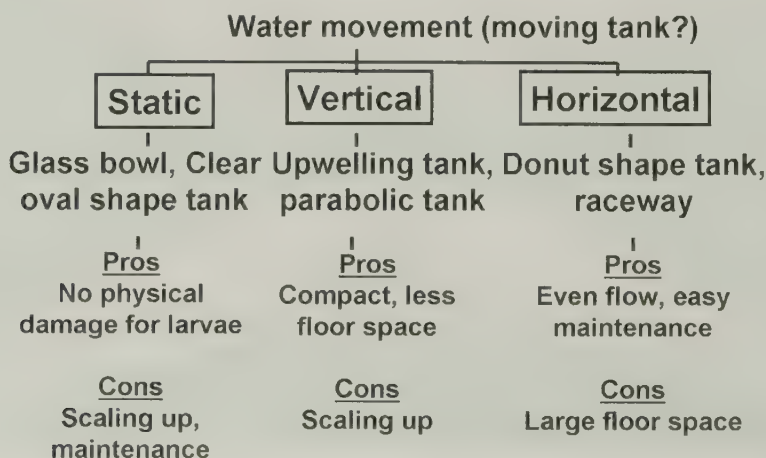


Fig. 3. The summary of larval rearing systems.

bacterial growth in the laboratory, they are not considered a long-term solution for the large scale rearing of lobster larvae. Alternative disinfection methods, such as UV and Ozone (O₃), should be considered.

Tank design with consideration of hydrodynamics is crucial for the maintenance of fragile phyllosomas. Because of the phyllosomas' unique morphology (flat body and long appendages), strong aeration can damage body segments. No water movement, or only gentle water movement, can be used in the tank. Movement of rearing water also needs to take into consideration the phyllosomas' behaviours (swimming, feeding and phototactic), food distribution and maintenance of tank system (Fig. 3).

Development of *Thenus* recirculation aquaculture facility in northern New South Wales, Australia

Despite the advantage of a short growout phase, there has been no record of commercial production of *Thenus* anywhere in the world. The major hurdle in the commercialisation of *Thenus* aquaculture has been the difficulty in maintaining the phyllosoma stages. Recently however, the scientific riddle of

growing Bay lobsters in a laboratory from eggs to juveniles was solved, with consistent survival of over 80 %. A pilot system for growing Bay lobsters has been operating successfully, and Australian Bay Lobster Producers (ABLP) is currently proposing to develop the aquaculture facility for the production of the *Thenus* spp. in northern New South Wales, Australia. The proposed facility will be based on recirculation aquaculture technology with animals housed in shallow raceways. The project has been designed to produce two products; live hard shell animals of an average 218 g and soft shell animals (frozen or fresh chilled) of an average 45 g. The proposed yearly production of Bay Lobster will be 1076 tonnes in Stage 1 and this will require a standing stock of approximately 400 tonnes of animals (Fig 4).

Australian Fresh Research and Development Corporation (AFR & DC) have been conducting research and pilot scale production of *Thenus* spp. over the past 11 years at the Department of Primary Industries & Fisheries, Bribie Island Aquaculture Research Centre (BIARC). During the early period of research bacterial disease of larvae, associated with poor nutrition was the identified

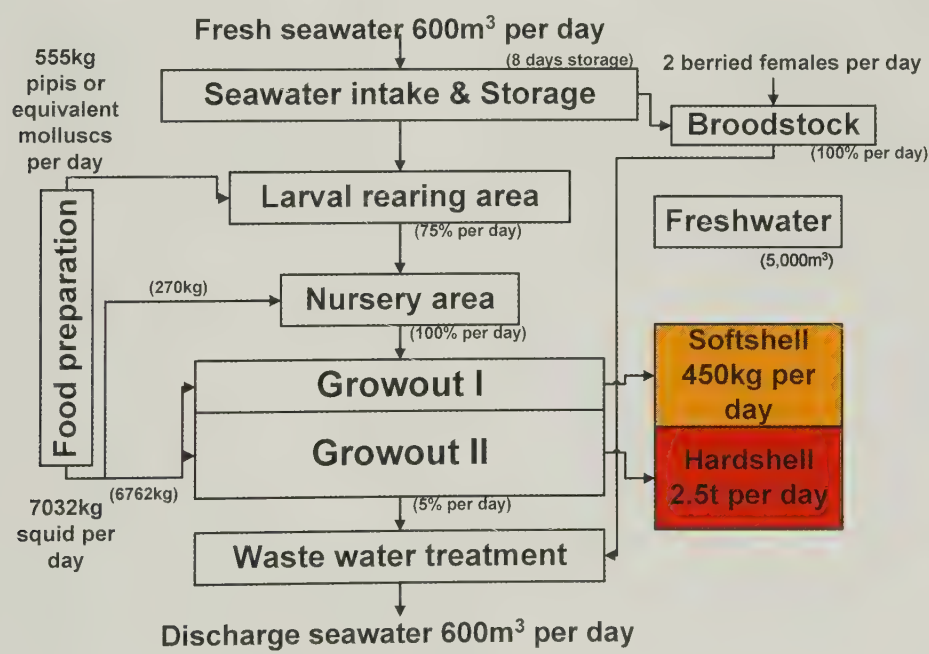


Fig. 4. The summary of proposed *Thenus* aquaculture facility in northern New South Wales, Australia.

cause of mortalities experienced. Modifications to the larval production system and optimization of nutritional requirements have dramatically reduced the incidence of bacteria-related mortality. After the larvae metamorphose to the nisto stage and then become juveniles, they become much more robust and no disease agents have been identified as causing mortality of juveniles/adults during culture trials. The bacteria that have been observed to infect phyllosomas are those always present in any body of seawater. The theory is these bacteria opportunistically attack phyllosomas that are in a poor state of health due to suboptimal environmental and nutritional conditions. Dosing of ozone to ensure sterilisation of the seawater can minimise the level of bacteria-related mortality.

Acknowledgement

The author would like to express his thanks to the member of workshop organizer, including Drs Sakai, Yokoyama, Awaji, Aono of the National Research Institute of Aquaculture and H. Matsuda of Mie Prefectural Science and Technology Promotion Centre. Special thanks are extended to Drs Akiyama, Nishimura and Tsujigado for their initial efforts being the workshop possible.

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Fisheries management of the spiny lobster *Panulirus japonicus* in Japan

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Abstract Among the palinurid lobsters caught in Japan, *Panulirus japonicus* is the most abundant and commercially important for fisheries. Coastal fisheries management system in Japan is characterized by the community-based management by fishermen organized in the fisheries cooperative associations (FCAs) which are exclusively granted coastal fishing rights under the authority of prefectural government. The management system in FCA is mainly based on spontaneous, self-motivating rules by fishermen, as well as compliance with the prefectural ordinance. Thus co-management of fisheries resources coordinated by fishermen and the government functions effectively as a fundamental framework. Management measures for lobster fisheries largely depend on “input control” and “technical measures”. They include: establishment of closed season or closed areas, introduction of various constraints for gear (type of nets, mesh size, thickness or quality of the net yarn, number of nets per boat), fishing recess around new-moon nights, rotation system of closed areas, limitation for allowable body size for landing, release of berried females or small individuals *etc.* In recent years, efficacy of the conventional management measures has been reinvestigated based on scientific management models, and some gaps between optimal measures and the actual ones are pointed out in some cases. A management model based on the optimal in-season allocation of fishing effort considering the shifts in market price, catchability, operation costs *etc.* was presented using the theory of maximum principle. Also, an optimal fishery model, which aims to integrate the effective utilization of recruited stocks and the reproductive management, was proposed by introducing a concept of economic value of the spawning stock. More detailed scientific examination should be intended to clarify the efficacy and the mechanism of the traditional management measures derived from the spontaneous activities by fishermen.

Key words: spiny lobster, *Panulirus japonicus*, fishery management

Novel molecular approach to study moulting in crustaceans

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Abstract Moulting occurs in all arthropods, from insects to crustaceans, it is essential for growth, reproduction and metamorphosis. Moulting occurs in cycles and involves the shedding of the hard exoskeleton to expose a soft new shell, the uptake of water from the animals' immediate surroundings causing the new exoskeleton to expand, and finally the hardening of the new exoskeleton. Moulting is a complex process that is affected by a range of external factors such as temperature, photoperiod, nutrition and eyestalk ablation. However despite extensive research the moulting process in crustaceans still remains poorly understood. Microarray technology provides a powerful, holistic approach to study gene expression in relation to changing physiological states. It enables not only the ability to profile the expression of genes already known to be involved in moulting, but also facilitates the discovery of new, as yet unknown, genes that may be important in the moulting process.

Understanding, and consequently controlling, the process of moulting, has significant potential for a range of commercial applications in crustaceans, such as the propagation of valuable seafood products. There are three areas within moulting control that have been identified as having potential commercial significance:

1. controlling the timing of the moult
2. manipulating the synchrony of moulting within a population (mass moulting)
3. controlling the process of shell hardening.

Key words: moulting, crustacean, microarray

Introduction into crustacean moulting

Moulting is common to all crustaceans and is essential for growth, metamorphosis and reproduction. Moulting is a complex process, affected by a range of environmental cues and regulated by a cascade of hormonal signals involving changes in gene expression, cellular commitment, mitotic and secretory activity, endocrinology, behaviour and cell death (Loeb, 1993).

The moult cycle refers to the period between two successive moults and has been subdivided into 4 major stages (Drach, 1939). These are known as the moult (E), postmoult (A-B), intermoult (C), and premoult (D) (Table 1). The moult stage, referred to as ecdysis, involves the shedding of the exoskeleton through a rapid uptake of water or air from the

environment, causing the exoskeleton to rupture. During postmoult, or metecdysis, further water uptake expands the new, still soft, exoskeleton; this expansion is essential for the growth of the animal. Exoskeleton mineralisation and hardening then occur. The intermoult period, or anecdysis, is the so-called period of non-activity, and by far the longest stage of the moult cycle. During this time, muscle regeneration occurs, and energy reserves such as glycogen and lipids are accumulated in the hemolymph and midgut for the succeeding moult. Premoult, or proecdysis, sees the atrophy of somatic muscle, the resorption of the old exoskeleton, and the formation of a new exoskeleton in preparation for the onset of ecdysis.

Two types of endocrine organs are associated with the moulting process, traditional androgenic

Received: November 25, 2005

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Table 1. The stages of the moult cycle*

STAGE	DESCRIPTION	DURATION	% DURATION
A	Newly moulted		
A1	Exoskeleton is a soft membrane; Crab is inactive; water absorption continues; exocuticle mineralisation begins	8 – 12 hrs	1
A2	Exoskeleton feels like parchment; crab begins to move about; water content stabilises to ~ 86%; endocuticle deposition and mineralisation begin (secretion of the postecdysial procuticle)	1 – 2 days	2
B	Recently moulted		
B1	Exoskeleton is deformable without breaking	1½ – 3 days	3
B2	Parts of exoskeleton become rigid; feeding may start	2½ – 4 days	5
C	Intermoult		
C1	Carapace almost completely rigid; main period of tissue growth	4½ – 5 days	8
C2	Carapace completely rigid; tissue growth continues	7 – 10 days	14
C3	Continuation of endocuticle mineralisation	7½ – 11 days	15
C4	Inner membranous layer complete – should remain attached to the epidermis; tissue growth complete; accumulation of metabolic reserves; water content 61%	15 – 22 days	30 +
C4T	Terminal anecdysis; animal is fully grown;		
D	Premoult		
D1	Epidermis separates from the membranous layer and secretes a new epicuticle; reserves are mobilised; glycogen builds up in the epidermal tissues; atrophy of somatic muscle; regeneration of autotomised limbs	3½ – 6 days	8
D2	New exocuticle secretion begins; old membranous layer degenerates in to a gelatinous layer; resorption of the old exoskeleton begins; reduced activity; feeding stops as the crabs loses its muscle insertions	3½ – 6 days	8
D3	Main period of old exoskeleton resorption	1½ - 3½ days	4
D4	Resorption of old exoskeleton is complete; old exoskeleton splits along epimeral lines; water uptake begins	12 – 15 hrs	1
E	Ecdysis		
	Animal withdraws from old exoskeleton and takes up water rapidly	Few minutes	

*Moult cycle stages based on (Drach, 1939) for *Cancer pagurus*. Durations based on (Hiatt, 1948)

glands, and neurosecretory cells, which are specialized neurons that form part of the central nervous system (CNS). The Y-organ and mandibular glands are examples of androgenic glands, they synthesize and secrete ecdysteroids and methyl farnesoate (MF) respectively (Claerhout *et al.*, 1996). Ecdysteroids are steroidal moulting hormones that are responsible for the growth, development, and reproduction of arthropods (Spaziani *et al.*, 1989). MF has also been implicated in the regulation of crustacean morphogenesis, metamorphosis, reproduction and moulting (Laufer *et al.*, 1993; Abdu *et al.*, 1998; Laufer and Biggers, 2001). Studies into ecdysteroid synthesis have shown that MF directly stimulates the secretion of ecdysteroids in *Cancer magister* Y-organs (Tamone and Chang, 1993; Chang *et al.*, 1993). Regulation of MF synthesis by the mandibular organ is negatively controlled via Mandibular Organ Inhibiting Hormone (MOIH), a neurohormone produced in the eyestalk (Liu *et al.*, 1997; Chaves, 2001).

The neurosecretory cells of the crustacean eyestalk are collectively termed the Sinus gland/X-organ complex (SG/XO). This is the primary negative regulatory system in crustaceans and is the site of synthesis and storage for the Moulting Inhibiting Hormone (MIH). As MIH negatively regulates ecdysteroid production by the Y-organs, precocious moults may be induced by eyestalk ablation. This results in a reduction of circulating MIH and therefore the immediate rise of ecdysteroids in the haemolymph. While eyestalk ablation is effective at inducing moulting, it also leads to lethal ecdysis in some species (Wheatly and Hart, 1995).

Novel molecular approach to study moulting in crustacea

In spite of extensive research investigating the physiological process underlying moulting, there is still no clear understanding of the cascade of events that regulate this process. Classical molecular approaches have focused on genes specifically related to moulting but have failed to comprehensively cover this complex process. New and powerful technologies such as microarrays offer a holistic approach to gene discovery and the study

of gene function in relation to changing physiological states (i.e., moulting). In order to explore the full set of genes involved in the moulting process, microarray technology has been implemented to investigate differential gene expression in *Portunus pelagicus* at various stages of the moult cycle. This has enabled both assessment of expression profiles of known genes, and the discovery of new genes that play a role in the moult cycle of crustaceans. *P. pelagicus* (commonly known as the blue swimmer crab) was used as a model species to study moulting due to its similar morphology to *Callinectes sapidus* (a highly valued soft shell seafood product in the United States of America), and due to hatchery technology developed at the Bribie Island Aquaculture Research Centre (BIARC) which allows for its complete culture in captivity, eliminating the need for wild caught animals.

Methods

P. pelagicus individuals were moult staged by examination of pleopod paddles for epidermal retraction and grouped into the following moult stages; moult (shedding of the exoskeleton), postmoult (pliable exoskeleton), intermoult (hard exoskeleton with no evidence of epidermal retraction) early and late stage premoult (based on the extent of epidermal retraction) (Freeman and Perry, 1985).

P. pelagicus microarray chips were custom printed by AgGenomics (Melbourne, Victoria, Australia), using cDNA libraries created from whole crablets (juvenile crabs with a 3-5 cm carapace width) in each of the five moult stages discussed above, and from the following crab organs brain, eyestalk, mandibular gland, Y-organ dissected from adult crabs in each of the five moult stages.

Experiments on the microarray chips were carried out to assess moult cycle related differential gene expression. This was done by hybridising cDNA labelled with Cy 3 (green) and Cy 5 (red) fluorophores to the chip. The cDNA used to probe the slides was isolated from whole crabs in the following moult stages: Moult, Postmoult, Intermoult, Early Premoult and Late Premoult. An Arrayworx scanner was used to scan the slides and data analysed via the

Biodiscovery software package, which includes Imagene for detecting signal intensity and spot morphology and Genesight for expression pattern analysis.

Results and Discussion

Previous studies describe the stimulatory effects of MF on ecdysteroid secretion (Tamone and Chang, 1993; Chang *et al.*, 1993). Farnesoic acid O-methyltransferase (FaMeT) is the enzyme responsible for the conversion of farnesoic acid (FA) to MF in the final step of MF synthesis. In this study FaMeT was found to be highly up-regulated in the intermolt stage of the moult cycle. This up-regulation implicates the direct involvement of FaMeT and indirectly MF in initiating the moulting process in crustaceans.

Synthesis and hardening of a new exoskeleton are essential to the arthropod moulting process. Cuticle proteins previously identified in calcified regions of the exoskeleton and those from flexible arthrodial membranes (soft cuticle at the joints) (Andersen, 1999; Wynn and Shafer, 2005) were found to be highly up-regulated in the moult and postmoult stages of the moult cycle indicating that these proteins are necessary for the formation and hardening of the exoskeleton. Cuticle proteins are suggested to be involved in the calcification process (Andersen, 1999; Kragh *et al.*, 1997) and in chitin binding (Wynn and Shafer, 2005). Another gene associated with exoskeleton formation is cryptocyanin, the expression of this gene was found to be highly up-regulated in the premoult stage of the moult cycle. Crustacean cryptocyanin belongs to a suit of hemolymph proteins, and is thought to transport hormones, phenols and/or some cuticular proteins to the arthropod hypodermis during premoult (Terwilliger, 1999), where they may become directly incorporated into the new exoskeleton (Terwilliger *et al.*, 1999; Haunerland, 1996). Metallothionein, also a hemolymph protein, was found to be up-regulated in the premoult and postmoult stages. Metallothionein is involved in copper homeostasis associated with the degradation and synthesis of hemocyanin, prior to and post moult, respectively (Syring *et al.*, 2000).

Many interesting expression profiles of "new" as yet unidentified genes were also determined. This was done by comparing the expression profiles of genes already known to be involved in moulting, with those profiles of as yet uncharacterised genes. If unknown genes display expression profiles which are similar (or opposite) to the profiles expressed by genes known to be important to the moulting process, they are selected for further analysis.

Molecular studies aimed at investigating the hormonal regulation of moulting in crustaceans through new and powerful technologies such as microarrays may provide a path into methods of controlling the crustacean moult cycle. Such control would have a direct impact on the large scale / commercial production of a variety of soft shell high value crustacean species.

Acknowledgements

This work was funded by the Queensland Government through the Aquaculture Industry Development Initiative of the Department of Primary Industries and Fisheries. Special thanks go to the supervisors of this project Abigail Elizur, Tim Holton and David Merritt. I would also like to extend my gratitude to the workshop organizers including Dr Sakai, Dr Yokoyama, Dr Aono and Dr Awaji of the National Research Institute of Aquaculture and H. Matsuda of Mie Prefectural Science and Technology Promotion Centre.

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Aspects of the technology of phyllosoma rearing and metamorphosis from phyllosoma to puerulus in the Japanese spiny lobster *Panulirus japonicus* reared in the laboratory

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Abstract Japan has a long history of more than 100 years in the development of rearing technology for phyllosomas of the Japanese spiny lobster, *Panulirus japonicus*. The first attempt to rear newly hatched phyllosomas was made in 1899, and it took about 90 years to succeed in rearing them to the puerulus and juvenile stages. The Minamiizu Station of the National Center for Stock Enhancement, Fisheries Research Agency, started to develop its technology for rearing the phyllosomas of this lobster in 1989, when it succeeded in rearing juveniles in the laboratory for the first time, although researchers at the station anticipated that the task would be difficult. We divided the technological development into two subject areas: development of rearing methods and development of diets. We worked on issues such as methods of treating the rearing water, optimum water temperature for rearing, new devices for mass-rearing, and measures to prevent bacterial disease, and we also set up tasks such as exploring foods and developing artificial diets to replace the gonad of the blue mussel, *Mytilus galloprovincialis*. We primarily used rearing tanks with a capacity of less than 50 L. As the technology developed, we were able to gradually increase the numbers of juveniles produced each year from several tens in 1994 to a few hundred in 2005.

Because of recent advances in the technology covering the period from hatching to metamorphosis into pueruli, we succeeded in observing 270 processes of metamorphosis in 2005, using final-stage phyllosomas reared for 228 to 429 days after hatching. The process of metamorphosis from phyllosoma to puerulus was divided into five stages: contraction of eyestalks, contraction of pereopods, molting of pereopods, molting of abdomen, and molting of antennae. We also clarified the apparent characteristics of metamorphosis and the time required for metamorphosis at each stage, and we revealed that the metamorphosis process took about 10 min from the very beginning to the end.

Key words: *Panulirus japonicus*, phyllosoma, rearing technology, metamorphosis, puerulus

Japan has a long history of artificial rearing of phyllosomas of the Japanese spiny lobster, *Panulirus japonicus*, and a rearing trial of newly hatched phyllosomas was first reported in 1899, more than 100 years ago (Hattori and Oishi, 1899). In 1958 phyllosomas were reared until they grew into third instars by being fed *Artemia salina* nauplii (Nonaka *et al.*, 1958), and in 1981 they were reared to the final stage immediately before metamorphosis into

pueruli by being fed *Artemia*, arrow worm (*Sagitta* spp.), and fish larvae and juveniles (Inoue, 1981). In 1989 Japan succeeded in rearing phyllosomas up to pueruli and lobster juveniles for the first time in the laboratory (Kittaka and Kimura, 1989; Yamakawa *et al.*, 1989). Thus 90 years of research elapsed before newly hatched phyllosomas were reared successfully for the first time. It was the Mie Prefectural Fisheries Research Station (currently the

Received: November 25, 2005

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Mie Prefectural Science and Technology Promotion Center - MPSTPC) and Kitazato University that first succeeded in rearing phyllosomas to the juvenile stage. MPSTPC used stagnant conditions, whereas Kitazato University took advantage of a closed recirculating system. Both institutions utilized *Artemia* and gonads of the blue mussel, *Mytilus galloprovincialis*, as food, and succeeded in rearing phyllosomas up to pueruli and juveniles.

The Minamiizu Station of the Japan Sea Farming Association (JASFA), which is currently called the Minamiizu Station of the National Center for Stock Enhancement, Fisheries Research Agency (NCSE, FRA) was established in 1989, the same year in which artificial rearing of phyllosomas up to juveniles was first reported. JASFA started to move on technological development to establish a mass-culture technology for Japanese spiny lobster phyllosomas. This station has specified rearing methods and natural and artificial diets as its two research subjects. In 2000 Minamiizu station joined a project team to develop a seed-production technology for decapod crustaceans; this project was designed to develop a mass-production technology for rearing

10 species of decapod crustaceans under study at seven out of the 16 stations of NCSE, FRA. In the initial stages of the research our station produced only a few pueruli and juveniles. Subsequently, as a result of technological development, the number of juveniles produced in our laboratory each year gradually increased to over 50 in 1994 and to over 200 in 2005 (Fig. 1). In addition, the survival rate from puerulus to juvenile stage was 30% to 50% before 2001, but it stabilized at a high level (60% to 80%) after 2002 (Fig. 1).

Here, we summarize both the technological aspects of phyllosomal rearing developed at our station and the process of metamorphosis, which we clarified during the development of a technology for mass rearing of final-stage phyllosoma larvae.

Technology of phyllosoma rearing at Minamiizu Station, NCSE, FRA

The technological development of lobster phyllosoma rearing has been extremely hard, for the following reasons:

- 1) The larval period lasts for nearly a year: the

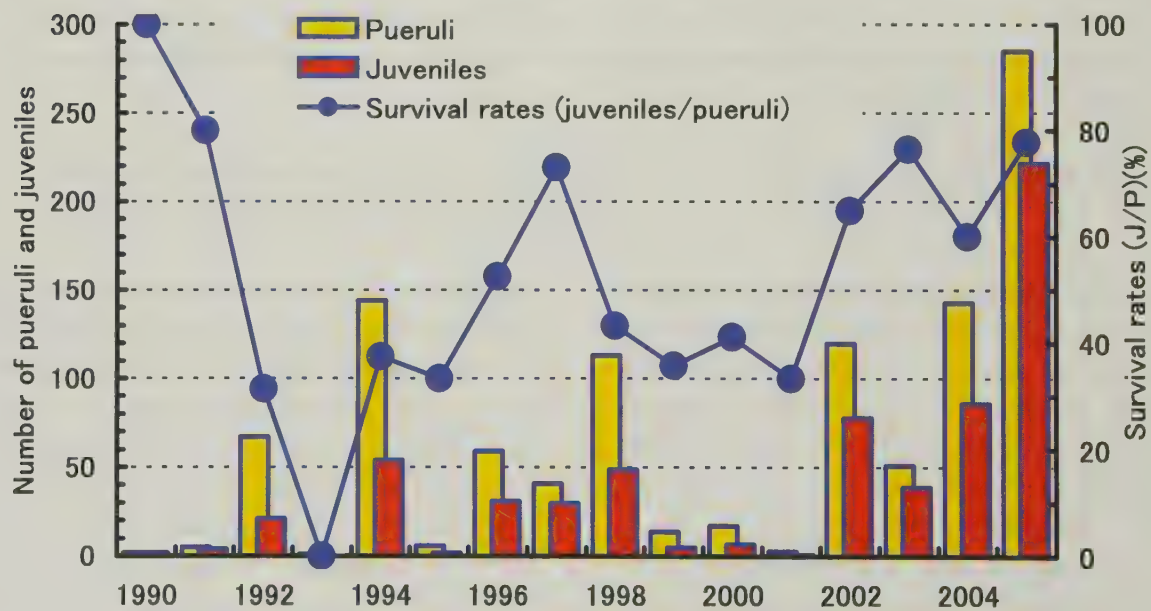


Fig. 1. Numbers of pueruli and juveniles and survival rates from pueruli to juveniles of *Panulirus japonicus* produced at Minamiizu Station, NCSE, FRA. J, juveniles; P, pueruli.

lobster grows from about 1.5 mm to 30 mm in body length (BL) from hatching to final-stage phyllosoma.

2) The larva has a transparent and flat body form and extremely long maxillipeds and pereopods. Because of this extraordinary form, larvae are liable to injure their pereopods and body surfaces through contact with other larvae.

3) The water temperature suitable for rearing is as high as 24 to 27°C, at which temperature larvae are prone to infection with bacterial diseases.

4) The only food that can be used to rear phyllosomas up to the puerulus stage is the gonad of the fresh blue mussel.

Since it was established in 1989, the Minamiizu Station of NCSE, FRA has been carrying out research on the following two subjects to solve these difficult problems.

Development of technical skills to rear phyllosoma larvae

We have been researching the following technological factors to allow stable rearing of phyllosomas: methods of treating water for rearing; appropriate water temperatures; development of rearing tanks; and measures to prevent bacterial

disease.

We tried three ways of pretreating the seawater used for rearing of phyllosoma: filtration by sand, filtration by 0.2- μ m hollow fiber membrane that could physically eliminate bacteria; and sterilization by ultraviolet irradiation after filtration by simplified 0.45- μ m cartridge filter. Comparative analysis of the three methods revealed that filtration by sand allowed larvae to survive for about 30 days after hatching, but the other methods enabled them to grow to the puerulus stage (Fig. 2). These results indicated the need to rear phyllosomas in clean seawater from which as many bacteria as possible had been eliminated. We also found that addition of antibiotics to the rearing water was essential to prevent bacterial disease, horizontal infection, and contamination of the body surface by bacteria while the phyllosomas were being reared.

After preliminary testing of several water temperatures to clarify which was best for rearing phyllosomas, we used rearing water at 24 and 27°C to compare growth to the puerulus stage. At 27°C all phyllosomas had died by about 220 days after hatching, whereas at 24°C they grew to pueruli (Fig. 3). These survival curves crossed between 140 and

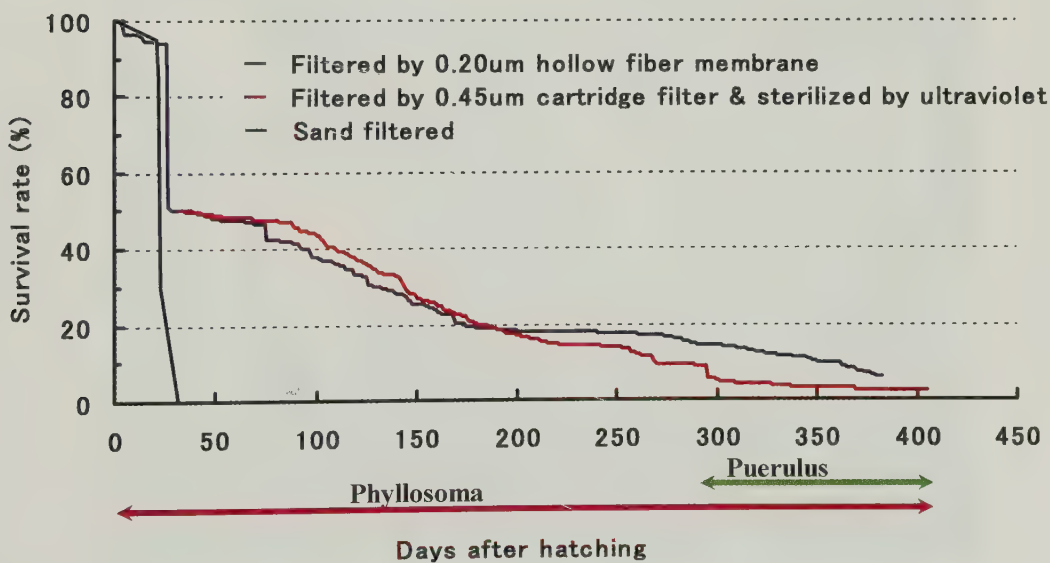


Fig. 2. Survival rates of *Panulirus japonicus* phyllosomas reared in 50-L acrylic semispherical tanks containing seawater treated as follows: filtration by hollow fiber membrane (pore size: 0.20 μ m), sterilization by ultraviolet after filtration by cartridge filter (pore size: 0.45 μ m), and filtration by sand.

150 days after hatching. We therefore concluded that 27°C was appropriate for the period 140 to 150 days after hatching and 24°C was appropriate after that period. This water temperature setting is still our standard for rearing phyllosomas.

The basic approach to developing a rearing tank is to construct a system that, by taking into account the species' body form and feeding behavior, allows efficient feeding and prevents the phyllosomas from damaging their pereopods and other body parts. Efficient feeding increases the chances of encounters between the phyllosomas and the food and prevents the loss of pereopods. In particular, it is necessary to decrease the degree of contact among phyllosoma larvae just before and after molting. A tank with a spherical bottom, which Nonaka and Inoue had already tried in the 1960s (Nonaka, personal communication), had been found to be effective in decreasing contact and dispersing phyllosoma larvae. Our station modified this tank design to create a bowl-type flow-through tank (600 mm in diameter, 500 mm high, actual capacity of 50 L; Fig. 4; Sekine, 1995), and we continued our rearing experiments using this type of tank. It thus became possible to rear many phyllosomas from hatching to puerulus, although the rearing results still lacked stability

(Sekine *et al.*, 2000). Nevertheless, this flow-through tank is still the standard for rearing phyllosoma. Fresh gonad of the blue mussel is an essential food for rearing phyllosomas up to the puerulus stage. Because small pieces of mussel gonads fall to the bottom of the tank, the conventional method creates a situation that allows phyllosomas to pick them up and eat them accidentally. In 2000, we started to develop a vertically revolving rearing system (VRR System; 300 mm wide, 600 mm in diameter, actual capacity 70 L; Fig. 5) that could create water flow of any strength inside the tank by revolving the whole rearing tank vertically; the aim was to increase the chances of encounters between the phyllosomas and the small pieces of mussel gonads (Murakami, 2004a). We used the VRR system to rear phyllosomas on an experimental basis and found that it encouraged their swimming activity; the survival rate to pueruli almost tripled in a few cases (Murakami, 2004a, b). However, this early-type VRR system tended to increase the number of contacts between middle- and late-stage phyllosomal larvae of more than 15 mm BL and to thus increase the number of phyllosomas with injured pereopods. Improvement is still under way.

Bacterial diseases that occur during the rearing of

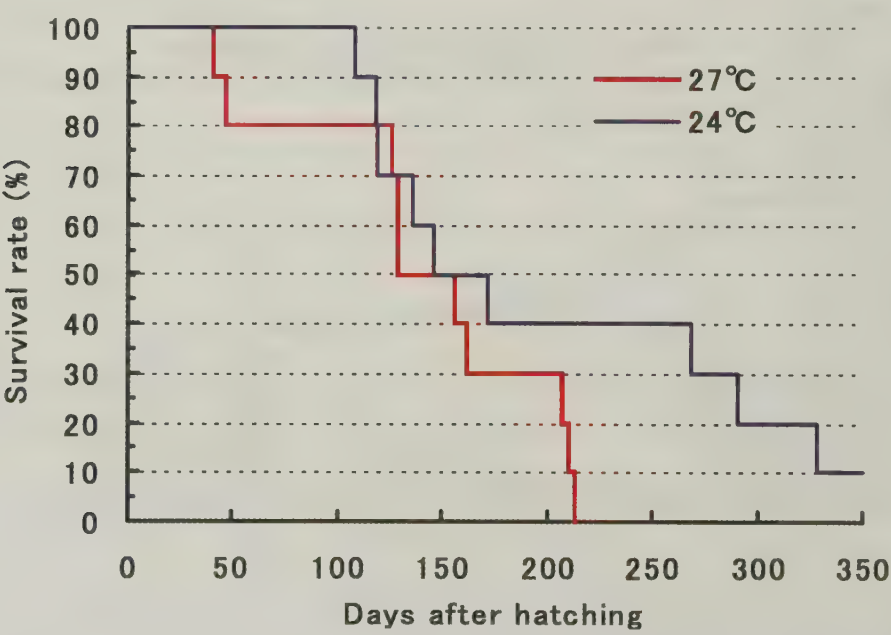


Fig. 3. Survival rates of *Panulirus japonicus* phyllosoma larvae reared in 1-L glass bowls at 24°C or 27°C .



Fig. 4. An acrylic semispherical tank with a volume of 50 L. This is the type used mainly for *Panulirus japonicus* phyllosoma rearing.

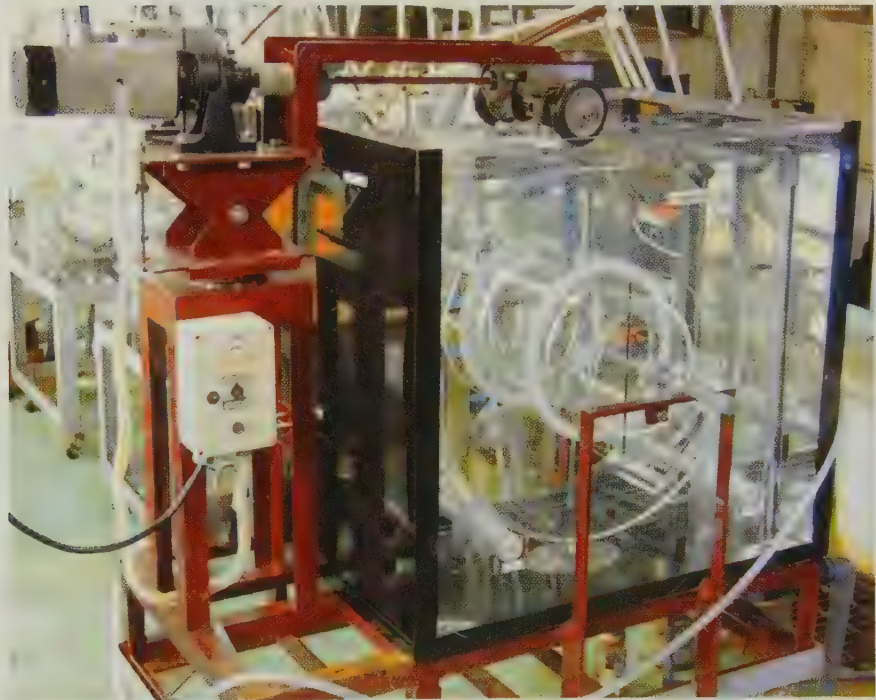


Fig. 5. The vertically revolving rearing system (VRR System), newly designed for *Panulirus japonicus* phyllosomas.

phyllosomas cause increased opacity due to necrosis of such parts as the antennal gland, hepatopancreas, hindgut, mandibles, maxillae, maxillipeds, and pereopods, in addition to incomplete molting. The latter is caused by the filamentous bacterium *Leucothrix mucor*, which presumably originates from the egg and becomes implanted on the body surface of the phyllosoma. Use of an antibiotic-medicated bath is effective in preventing secondary infection with these diseases. It is effective to immerse the larvae in streptomycin sulfate or ampicillin 10 ppm for at least 15 h to prevent filamentous bacterial disease, which occurs predominantly in early-stage phyllosomas. Immersion of the phyllosomas in chloramphenicol 10 ppm for 24 h was effective in preventing increased opacity of the maxillipeds, pereopods, and hepatopancreas. Furthermore, such bacterial diseases could be prevented effectively by making it a rule to change the rearing tank and immerse the phyllosomas in ampicillin 20 to 40 ppm for 15 to 18 h about once a week (Murakami and Hashimoto, 2003a). Under this rearing protocol, the number of bacteria in the rearing water can be expected to decreased to $<10^3$ CFU/mL (Murakami, 2004b). Adding antibiotics to the rearing water is essential for rearing phyllosomas from hatching to juveniles.

Exploitation of foods for phyllosoma larvae

Artemia enriched by the diatom *Phaeodactylum tricornutum* is used alone to feed phyllosomas up to 30 days after hatching; the gonad of the blue mussel, together with grown *Artemia* whose size is adjusted by culture, is used after this larval period. At this point, it is impossible to rear phyllosomas to pueruli only on *Artemia*, and only addition of the mussel gonad can enable them to grow into healthy pueruli.

To rear phyllosomas, we tried a total of more than 40 food species, including 15 species of zooplankton; five species of fish larvae and juveniles; the muscles, livers, and gonads of two crustaceans; six shellfish and three fish species; and the livers of cows and pigs (Sekine, 1999). We found that no food could be substituted for the gonad of the blue mussel in phyllosoma rearing. In feeding experiments tried recently, spontaneous ingestion of frozen sergestid shrimp (*Sergia lucens*) and frozen sand lance,

Ammodytes personatus, by late-stage phyllosomas made it possible to rear them for half a month to pueruli, but these pueruli did not live long enough to become juveniles (Murakami and Hashimoto, 2003a). We used frozen sergestid shrimp to replace the mussel gonad for rearing early-stage phyllosoma larvae, and we succeeded in rearing them for more than 250 days. However, the phyllosomas did not grow as well on frozen sergestid shrimp as they did on mussel gonad (Murakami and Hashimoto, 2003b). At present, the fact remains that the gonad of the blue mussel is still the best food for phyllosoma rearing (Murakami and Hashimoto, 2004).

We examined a few food liaisons to prepare artificial diets, but we found that artificial diets that were palatable were made mainly of sea urchin, *Hemicentrotus pulcherrimus*, or mussel gonad with 2% alginate sodium additive. Each of them could be used for short periods of about 2 months, but phyllosomas reared on either of them had considerably lower growth rates than those reared on fresh mussel gonad. Even now, we are making efforts to develop artificial diets that ensure stable supply in terms of volume and preservation for long periods.

Metamorphosis from phyllosoma to puerulus

Highly active final-stage phyllosoma larvae just before metamorphosis are seldom captured in the wild, because they inhabit the pelagic ocean and their long pereopods are easily injured by the sampling gear (Yoshimura, 2005). Similarly, it is extremely hard to rear large numbers of highly active final-stage phyllosoma larvae, as indicated by the results obtained during the long history of research on the rearing of phyllosomas. Therefore, until now, the process of metamorphosis from phyllosoma to puerulus had remained unknown. As a result of the recent improvements in the technology of phyllosoma rearing, in 2005 it became possible to rear relatively large numbers of final-stage phyllosoma larvae, and we succeeded in observing the metamorphosis process in detail under laboratory conditions.

We used 270 final-stage phyllosoma larvae reared for 228 to 429 days after hatching to observe their

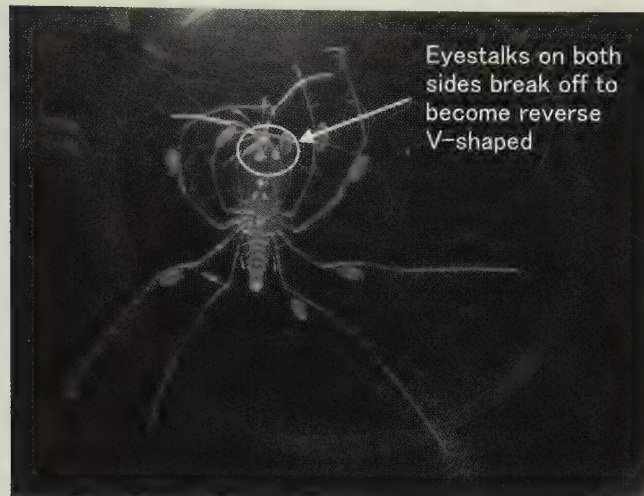


Fig. 6. Photograph of first stage (contraction of eyestalks) in the process of metamorphosis from final stage of phyllosomas of *Panulirus japonicus* to puerulus stage.

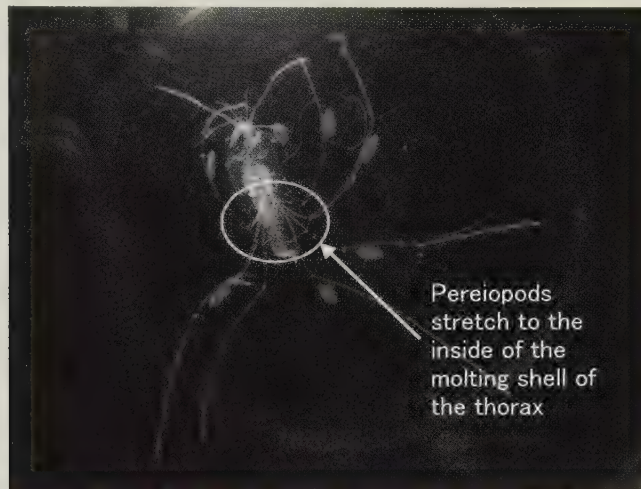


Fig. 7. Photograph of third stage (molting of pereiopods) in the process of metamorphosis from final stage of phyllosomas of *Panulirus japonicus* to puerulus stage.

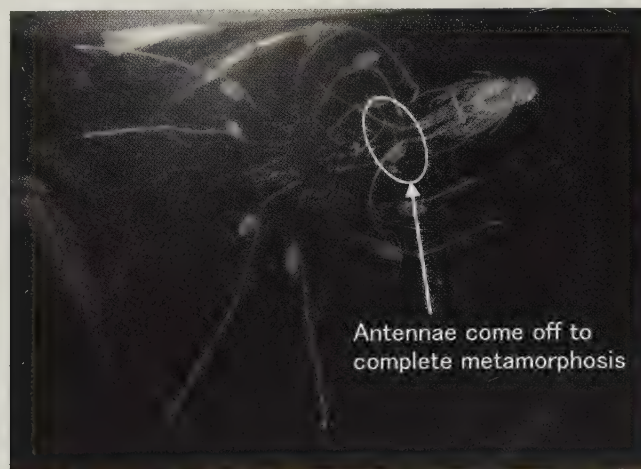


Fig. 8. Photograph of fifth stage (molting of antennae) in the process of metamorphosis from final stage of phyllosomas of *Panulirus japonicus* to puerulus stage.

metamorphosis to pueruli. These phyllosoma larvae became pueruli in 245 to 449 days after hatching; the average time taken was 319.5 days, with a standard deviation of ± 37.8 days. From the very beginning to the very end, the metamorphosis process could be divided into five stages of visible change: contraction of eyestalks, contraction of pereopods, molting of pereopods, molting of abdomen, and molting of antennae.

We were able to confirm the beginning of metamorphosis from the time when the V-shaped eyestalks gradually broke off at their bases toward the cephalon side to become linear. In the first stage (contraction of eyestalks), the eyestalks further break down toward the cephalon side to become a reverse V shape, and the motion of the exopodites stops (Fig. 6). In the second stage (contraction of pereopods) the pereopods contract to the bases of the exopodites, and the tips of the dactylopodites can be confirmed inside the molting shell. In the third stage (molting of pereopods) the pereopods completely molt inside the thorax shell and the thorax creeps inside the cephalon to form the cephalothorax of the puerulus (Fig. 7). The eyestalks that began contracting in the first stage contract further to the bases of the antennae to become V-shaped again. In the fourth stage (molting of the abdomen) the abdomen rises inside the molting shell of the thorax to make inroads between the cephalon and thorax, and the cephalothorax and abdomen of the puerulus spring outside the shell almost simultaneously to molt. In the fifth stage (molting of antennae) the puerulus jumps backward by its abdomen, and the eyestalks, antennules, and antennae molt, in that order (Fig. 8). When the antennae have finished molting, the process of metamorphosis to puerulus is complete. The total time required for metamorphosis from phyllosoma to puerulus was 10 min and 39 s ($N = 178$; standard deviation ± 2 min and 10 s).

Perspectives

Minamiizu Station of the NCSE, and the MPSTPC, are the only two institutions in Japan that are conducting research on the rearing of phyllosomas of the Japanese spiny lobster. The two institutions hold review meetings on a regular basis to deepen

their exchange and thus improve the technology of rearing phyllosomas. For the 2005-2008 plan, the FRA initiated a national project commissioned by the Agriculture, Forestry and Fisheries Research Council. Tokyo University of Marine Science and Technology and MPSTPC are participating in the project. They aim to develop an optimum food and to confirm suitable rearing conditions for the healthy growth of phyllosomas; they also aim to greatly increase the survival rate of phyllosomas from hatching to juveniles. Hopefully the fruits of this project will resolve the remaining problems and improve larval rearing technology in seed production of the Japanese spiny lobster.

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Lobsters do well in sea-cages: Spiny lobster on-growing in New Zealand

Phil JAMES*

Abstract The spiny red rock lobster (*Jasus edwardsii*) is the third-biggest seafood export earner in New Zealand with exports from the wild fishery valued at just under (\$NZ) 100 million in 2004. Consequently, there is significant interest in capturing wild *Jasus edwardsii* pueruli and on-growing them through to a marketable size.

Following several attempts to on-grow pueruli in land-based facilities the focus on lobster aquaculture research in New Zealand has switched from land to sea-based on-growing utilising specifically designed sea-cages.

Following experimental scale sea-cage on-growing, and in conjunction with commercial partners, NIWA have successfully run commercial scale sea-cage on-growing trials, rearing *Jasus edwardsii* pueruli from wild caught pueruli through to a marketable size of 200-250 g in 2-3 years. These trials culminated in the presentation of sea-cage on-grown lobsters to politicians and restaurateurs in December 2003. Following this commercial success NIWA has continued research into sea-cage design and the development of an artificial lobster diet to improve the economic viability of lobster on-growing in sea-cages.

This presentation describes sea-based lobster on-growing research in New Zealand and the relevance of this research to lobster aquaculture in other countries.

Key Words: spiny lobster, *Jasus edwardsii*, on-growing, sea-cages

The spiny red rock lobster (*Jasus edwardsii*) is the third-biggest seafood export earner in New Zealand with exports from the wild fishery valued at just under (\$NZ) 100 million in 2004. Consequently, there is significant interest in capturing wild *Jasus edwardsii* pueruli and on-growing them through to a marketable size.

NIWA has undertaken an extensive research programme over the past 10 years to investigate the optimal conditions for on-growing wild caught pueruli. Initially the focus was on land-based holding systems and measured the efficacy of natural diets (James and Tong, 1997; James, 1998; James and Tong, 1998) and the effects of a number of environmental factors on lobster growth and mortality. The research showed that the optimal stocking density for captive lobsters is 50 lobsters m²/internal surface area (James *et al.*, 2002). Subsequent commercial scale land and sea-based

trials have confirmed this research. The research also showed that the presence of shelters in a land-based holding system had no significant effect on lobster growth but lobsters that had shelters present had significantly better survival rates (James *et al.*, 2002). Subsequent sea-cage trials have shown that it is possible to substitute shelters for a complex 3-dimensional structure that both significantly increases the internal surface area of the holding system and provides shelter for the animals (Unpublished data). A study of the effects of salinity on lobster growth and mortality found that both fluctuating (25%-35‰) and low (25‰) salinity treatments had significantly lower growth and higher mortality than 30‰ and 35‰ treatments (Moss *et al.*, 2000).

A series of experiments studying optimal tank design for on-growing lobsters showed that long shallow trays are not as suitable as medium or deep

Received: November 25, 2005

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tanks and that it is possible to increase the density of lobsters held in deeper tanks by simply increasing the internal surface area available to the lobsters (James *et al.*, 2003).

In 2001 an economic study was undertaken to establish the commercial viability of on-growing lobsters in a land-based facility. This study showed that economic land-based lobster on-growing would 'rely on greatly reducing the infrastructure and operating costs of land-based operations and lowering feed and labour costs' (Jeffs and Hooker, 2000) and that sea-based on-growing offered a more viable option for lobster on-growing. Subsequently, following several commercial attempts to on-grow pueruli in land-based facilities, the focus on lobster aquaculture research in New Zealand has shifted from land to sea-based on-growing.

A sea-cage on-growing experiment showed that animals held in sea-cages in the north of New Zealand, where ambient temperatures are significantly warmer, had significantly higher growth rates than lobsters held at sites further south where ambient seawater temperatures are cooler (Jeffs and James, 2001). However, the mortality of the lobsters held at warmer temperatures was significantly higher than those held at cooler temperatures. Following the success of this experiment, and in conjunction with commercial partners, NIWA undertook a commercial scale sea-cage on-growing trial, rearing wild caught pueruli through to a marketable size of 200-250 g. The trial involved 2 geographically separate sites. One site was in the Coromandel in northern New Zealand where water temperatures are significantly warmer than at the second site in the Marlborough Sounds further south. The results of this study again showed that lobsters had significantly greater growth in the northern, warmer site but that the mortalities were also higher in the warmer temperatures. Lobsters were held at two separate study locations at the southern site and the results showed significant differences in both growth and mortality between the two locations, despite there being no difference in ambient water temperatures. The trials also clearly showed that lobsters held in the commercial sea-cages had significantly better growth than any previous land-based on-growing trials and that it was

possible to grow *Jasus edwardsii* to a commercial size in approximately 3 years (James and Jeffs, 2003; Jeffs and James, 2003). These trials culminated in the presentation of sea-cage on-grown lobsters to politicians, restaurateurs and lobster farmers in December 2003.

Following this commercial success, NIWA has continued research into both sea-cage design and the development of artificial lobster diets to improve the economic viability of lobster on-growing in sea-cages. During the commercial sea-cage trials there were a number of issues with cage design that were identified. These included problems with accessibility, ease of cleaning and feeding, and the requirement for heavy lifting gear to service the cages. The durability of the cages and the ability of the cages to retain early juvenile animals were also problematic. Consequently, NIWA has designed a new, open-topped design for on-growing lobsters in sea-cages. The design allows for easy feeding and access to the animals. Lobsters held in the cages in preliminary trials have shown very good growth rates and high survival compared to previous trials. Following this success of the sea-cage design in the preliminary trials NIWA has begun a commercial scale experiment to test the efficacy of the new cage design compared with the commercial cages that had been previously used. In addition, the efficacy of both natural, and artificial diets are being tested during the experiment using mussel, pellet and moist block diets. An additional treatment has been included to determine the effect on the growth of lobsters of the natural fouling that occurs on the cages.

The current sea-cage research at NIWA also aims to study the feeding behaviour of rock lobster juveniles in sea-cages. This will be monitored using remote video surveillance. The research will focus on the aggregation of lobsters at feed stations, aggression and formation of feeding hierarchies, frequency and periodicity of feeding. In addition, the consumption and digestion of the three diets will be measured in relation to the growth trials. The research will also focus on diet water stability, feed intake, feed-loss during manipulation, digestibility and gut retention time.

The research undertaken by NIWA has provided

critical information on optimal stocking densities, the effects of shelter and tank design and salinity. The research has shown that land-based on-growing is unlikely to be economic, but sea-cage lobster on-growing shows enormous potential. There are still a number of areas that require further research and these include sea-cage design, site selection and the availability of a suitable artificial diet.

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Morphological notes on the mouthparts of decapod crustacean larvae, with emphasis on palinurid phyllosomas

Kooichi KONISHI *

Abstract Some topics in the morphological study of mouthparts of decapod larvae, consisting of labrum, mandible, paragnath (= labium) and maxillule, are briefly reviewed. The morphological features of larval mouthparts are compared topographically in main decapod groups. Among them, two unique types are noted: palinurid and some thalassinid mouthparts. Palinurid phyllosoma larvae have dorsoventrally flattened mandibles sandwiched and covered between a large labrum and paired paragnaths. Furthermore, other posterior appendages, maxilla and maxillipeds, are placed far apart from the position of mouthparts. Phyllosoman mouthparts of *Panulirus japonicus* by VTR showed that active movements of labrum and paragnaths. Morphological characters of phyllosoman mouthparts suggest that microphagus feeding in their larval life based on Alexander's (1988) comments. In the symmetry of larval structures, two thalassinid families, Laomedidae and Thalassinidae, the zoeal larvae have remarkably asymmetrical mandibles and paragnaths. At present nothing is known on feeding mechanisms by those unique mouthparts. In addition, *lacinia mobilis* is found in left mandible of some caridean zoeal larvae and we have no information on the role of this structure which is lacking in adult phase of decapod mouthparts.

Key words: decapod crustaceans, mouthparts, mandible, morphology

In decapod larvae, the functional morphology of elementary mouthparts, consisting of labrum, mandible, paragnath (= labium) and maxillule, has been poorly understood in spite of many previous descriptive studies. One of the reasons for this condition may be due to technical difficulty in practical observation of mandibles-dissecting very small and fragile elements by hand, and no alternative, drastically improved methods have been developed to date. In addition, microscopical observations on the movement of each mouthpart for living small larvae have not been presented yet.

In this study, the larval mouthparts are compared topographically in main decapod groups. Among them, two unique forms are noted: palinurid and some thalassinid mouthparts, etc.

Palinurid mandibles

Palinurid phyllosoma larvae have dorso-ventrally flattened mandibles which are sandwiched between a large labrum and large paired paragnaths (Fig. 1A, 1B, arrow). In addition, other posterior appendages, maxillae and maxillipeds, are placed far apart from the position of mouthparts. Feeding motions of these parts have been fragmentally documented. Observation of mouthparts in the living 1st stage phyllosoma of *Panulirus japonicus* using VTR showed that active movements of labrum and paragnaths. Mastication mechanism in detail in digestive system of phyllosoman larvae is not confirmed yet, although Lemmens and Knott (1994) described the morphological change of mouthparts during metamorphosis in *Panulirus cygnus*. Alexander (1988) commented that the paragnaths

Received: November 25, 2005

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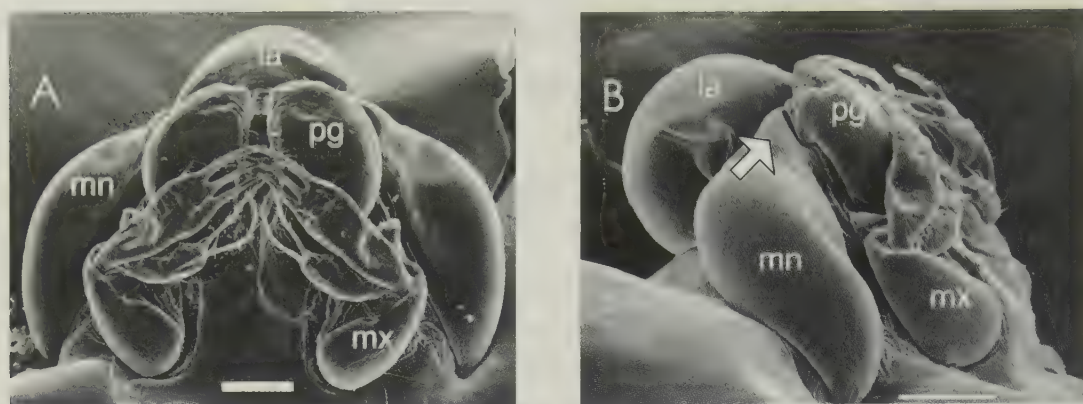


Fig. 1. Scanning electron micrographs of mouthparts of 8th phyllosoma in *Panurilus japonicus* in frontal (A) and lateral (B) view. White scale bars = 0.1 mm. la: labrum, mn: mandible, mx: maxillae, pg: paragnath.

are relatively small in macrophagus forms and show the greatest development in microphagus forms. Morphological characteristics of phyllosoma mouthparts suggest microphagus feeding mode in their larval life.

Heegaard (1969) described the mandibles of amphion larvae, having some similar body-plans to phyllosomas, and he stated that the two long lobes on the labium (= paragnath) are placed close together in phyllosoma, a reptant character, but in amphion they are short and placed far apart. Schram (1986) stated, "the mandibles are rather reduced and are typically formed as weak molar lobes, with no incisor lobe or palps. In females, the mandible have been termed vestigial, over which the labrum and paragnaths form opposed simple complex and concave flaps, respectively. The zoeal or amphion larvae and the males have well-developed mouthparts and guts. However, the reduced mouthparts and vestigial guts of the adult female indicate these do not feed but live off food reserved. Nothing is known about mode of feeding, or details of reproduction or locomotion". These morphological features suggest that the mandible of *A. reynaudii* is similar to those of the phyllosoma larvae of spiny lobsters except for topographic condition of labrum and paragnath.

Asymmetry in thalassinid mandibles

Another unique example is found in thalassinid

mouthparts. Among zoeal larvae of the infraorder Thalassinidea, so far as known, those of the family Laomediidae and Thalassinidae have considerably asymmetrical mandibles and paragnaths - the left one is extremely elongated, while right one is very small (Gurney and Lebour, 1939; Sankolli, 1967; Yaldwyn and Wear, 1972; Goy and Provenzano, 1978; Fukuda, 1982; Rodrigues *et al.*, 1992). Many previous authors have been commented this uniqueness (Gurney, 1942; Factor, 1989), but no observations for movement of the mouthparts have been made to date. We have, therefore, no idea for actual function of the asymmetrical mandibles. Asymmetry in larval mouthparts has been known in some insect groups (cf. Heming, 1980), but there are no ideas for functional or adaptive significance for this just until recent years. Inoda *et al.* (2003) firstly revealed the function of asymmetrical mandibles of some larval insects-adaptation for catch and hold of the prey. In near future, we will make it clear about this interesting structure by advanced observation techniques of living larvae.

P.S. Lacinia mobilis in some decapod larvae

In addition to above two cases, special small and articulated seta-like structure on incisor process of mandibles, lacinia mobiles, should be noted. This minute spine-like structure has been known in adult form of peracarid decapods (cf. Dahl and Hessler, 1982; Richter and Kornicker, 2006). In some

caridean zoeal larvae (e.g., Konishi and Kim, 2000), the left mandible possesses this structure while right mandible not. In adult peracarid shrimp, some possible roles in feeding prey have been stated, but no suggestions for decapod zoeas.

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Development of technology for larval *Panulirus japonicus* culture in Japan: A review

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Abstract Of six *Panulirus* lobsters that occur in the shallow waters of Japan, the Japanese spiny lobster *Panulirus japonicus* is the most abundant and the most important to Japanese coastal fisheries. Hence, Japan has had a long and great interest in the propagation and aquaculture of this lobster. Research on larval (phyllosoma) lobster culture in Japan commenced in 1898, so there have now been over 100 years of research and development. However, specific biological characteristics of phyllosoma, such as their peculiar body form, protracted lifespan (ca. 1 year), and pelagic open-ocean life, have hindered significant progress in culture. The first complete culture from hatch to juvenile stage occurred in 1988 at Mie Prefectural Science and Technology Promotion Center, exactly 90 years after the first trial. Subsequently, the number of juveniles produced in the laboratory per year has increased gradually up to ca. 300 in 2003, a result that reflects the increasing availability of information on optimal culturing conditions, such as optimal environmental parameters, feeding, and tank design. Still, there are significant problems to overcome in the establishment of large-scale culture of phyllosoma. These further challenges include the control of bacterial diseases and excessive aggregation of larvae, the use of prepared diets such as artificial foods, and the reduction of high operating costs. At present, these problems are being examined in an effort to increase the number of larvae cultured in a single tank and to stabilize larval culture by controlling bacterial levels.

Key words: *Panulirus japonicus*, phyllosoma, culturing conditions, tank design

Introduction

The Japanese spiny lobster *Panulirus japonicus* is distributed along the central and southern Pacific coasts of Japan and in the waters of southern South Korea and northern Taiwan (Sekiguchi, 1997). This lobster is highly valued as seafood and holds an important position in the coastal fisheries of Japan; the total annual catch in Japan ranged from 969 to 1691 tonnes during the period 1951-2002 and generated US\$52 million in 2002. However, the lobster fishery is fully exploited (Yamakawa, 1997) and the development of aquaculture is required for further expansion of production. The greatest barrier to the establishment of aquaculture is the difficulty in producing large numbers of juveniles

through larval culture. Despite numerous efforts to improve the technology of larval culture, large-scale larval culture has not been achieved (Saisho, 1962, 1966; Inoue, 1981; Matsuda and Yamakawa, 1997; Sekine *et al.*, 2000). Factors that have hindered phyllosoma culture are:

- 1) the peculiar form of the body (extremely long and thin maxillipeds and pereopods)
- 2) the tremendously long life (almost 1 year)
- 3) frequent occurrence of bacterial diseases, probably because of the larval pelagic open-ocean life
- 4) induction of excessive aggregation as a negative photoreaction
- 5) availability of few food regimes and little information on optimal feeding conditions

Received: November 25, 2005

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6) lack of availability of information on ecology.

Although it has been difficult to overcome each of these factors, techniques of culturing phyllosoma are improving steadily but gradually, and a few hundred juveniles per year can now be produced in the laboratory.

Here, we give an overview of the development of phyllosoma culture in Japan and describe the optimal cultural conditions that have so far been elucidated.

Research on phyllosoma culture in Japan

Japan has had a long history of research on *P. japonicus* phyllosoma culture, exceeding 100 years. The development of this culture has been reviewed briefly by Nonaka *et al.* (2000). Recently, there were some remarkable advances that may lead to an expansion of the scale of culture. In this section, we overview the latest developments in larval culture.

Three major phases can be distinguished in the long history of this research. The first phase commenced in 1898 with a culture experiment carried out by Hattori and Oishi (Hattori and Oishi, 1899). Their experiment was simple: they cultured newly hatched larvae on diets of fish and lobster flesh and kept the larvae in a still-water

system in small tanks. Then Mie Prefectural Fisheries Research Station (the present Mie Prefectural Science and Technology Promotion Center-MPSTPC) put newly hatched larvae into small boxes, around which cotton screens were fixed, and cultured them without feeding (Fig. 1) (Anon, 1934). The screens prevented the larvae from escaping, but allowed entry of photoplankton and small zooplankton, which the researchers expected would be food for the larvae. Other trials were conducted at many fisheries laboratories using similar simple equipment until the mid-1950s, but in all these trials the larvae did not survive long enough to molt to the second instar. This primitive phase in the development of phyllosoma culture can be referred to as Phase I.

Phase II began in 1957 with successful culture from hatching to subsequent instars by the feeding of *Artemia* nauplii (Nonaka *et al.*, 1958). After this success, research on phyllosoma culture became even more active (*e.g.*, Saisho, 1966; Inoue, 1981). Saisho (1966) obtained a larva of 6.4-mm body length (BL), and Inoue (1981) succeeded in producing a gilled-stage larva of 29.64-mm BL, using a specially designed circular tank and the feeding of a combination of several diets, such as *Artemia*, natural zooplankton, and fish fry. Inoue's success was achieved by the introduction of many innovations to the culture system, such as changes to the tank design, seawater supply and drainage system and the use of a seawater current to maintain the larvae in suspension, as well as the use of large amounts of labor to collect natural zooplankton and many kinds of fish fry. However, research on phyllosoma culture then ebbed away because of the difficulties and huge amount of work involved and the continuing inability to produce pueruli and juveniles.

Significant progress in culturing the phyllosoma of palinurid lobsters was made in 1987-1988. Kittaka (1988) cultured larvae hatched from *Jasus lalandii* transferred from South Africa in closed-circulation systems, and successfully produced a puerulus for the first time in 1987. He achieved complete culture by feeding mussel gonad and introducing the microalga *Nannochloropsis oculata* into the culture seawater. Subsequently, by using methods similar to those used with *J. lalandii*, he and coworkers also

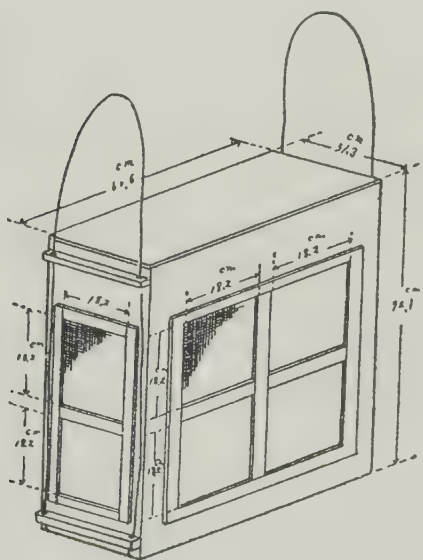


Fig. 1. Vessel for culturing phyllosoma of *Panulirus japonicus*, as used by the Mie Prefectural Fisheries Research Station in 1929 (Anon, 1934).

succeeded in producing pueruli of a hybrid between *Jasus novaeollandiae* and *Jasus edwardsii* (Kittaka *et al.*, 1988) and of *Palinurus elephas* (Kittaka and Ikegami, 1988).

Yamakawa *et al.* (1989) cultured about 1000 newly hatched larvae of *P. japonicus* in 1-L glass vessels under stagnant conditions, and they successfully obtained a juvenile lobster in 1988. Shortly after this success, Kittaka and Kimura (1989) obtained two juveniles. Phase III in the development of phyllosoma culture started with these successes in 1988. Thereafter, complete larval cultures of this species were achieved by the Tazaki Pearl Co., Ltd. (cited in Kittaka, 2000) and the Japan Sea Farming Association (Sekine, 1995). Information began to accumulate on the optimal culturing conditions, such as optimal environmental parameters and feeding, and on the biological and physiological aspects of larvae; this resulted in increasing survivorship in small-scale cultures in 100- to 1000-mL vessels (*e.g.*, Matsuda and Yamakawa, 1997; Matsuda *et al.*, 2003). When phyllosoma were cultured communally in larger tanks, however, rates of survival from hatching to the juvenile stage were still low because there was little information on the optimal conditions for culturing the middle and late stages in large tanks. Recently, the use of new devices for mass culture has been proposed (Murakami, 2004; Matsuda and Takenouchi, 2005), and the number of juveniles produced in the laboratory has been increasing gradually, up to ca. 300 in 2003 (Matsuda, 2004).

At present, research on the culture of *P. japonicus* larvae is under way at two research institutes in

Japan: MPSTPC and the Minamiizu Station, National Center for Stock Enhancement, Fisheries Research Agency. A national project aimed at further developing phyllosoma culture commenced in 2005, and other institutes joined the project to develop feeding methods and clarify optimal environments. This project is expected to produce further advances toward large-scale phyllosoma culture.

Phyllosoma culture technologies

There are many factors that affect phyllosoma survival and growth. These factors can be classified into four categories: environmental parameters, nutrition, culture management, and equipment and facilities (Table 1). This section will explain some of the factors that have marked effects on growth and survival.

Temperature

Temperature is one of the most influential and important factors in phyllosoma culture. Low temperatures can cause larval mortality, because the intermolt period is prolonged and consequently the larval body becomes tainted with foods given and debris floating on the surface of the culture tank. On the other hand, high temperatures induce deterioration of seawater and an imbalance between energy uptake and consumption. Hence, it is crucial to determine the optimal temperature for larval culture. Fig. 2 shows the relationship between larval growth efficiency (as calculated from the amount of carbon used for growth as a percentage of the total amount of carbon consumed by the larvae) and

Table 1. Factors that affect phyllosoma growth and survival

Category	Factor
Environmental parameters	Temperature; Salinity; Photoperiod; Light intensity; Water flow rate; Water current; Larval density
Nutrition	Kind and quality of food; amount of food; Food size
Culture management	Frequency of tank water change; Flow-through or re-circulation systems
Equipment and facilities	Tank design; Filtration and sterilization of seawater

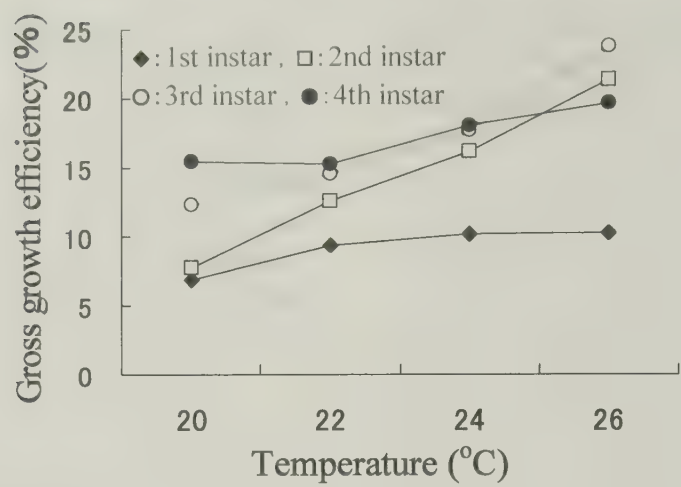


Fig. 2. Effect of temperature on gross growth efficiency (as calculated from the amount of carbon used for growth as a percentage of the total amount of carbon consumed by the larvae) of first to fourth instar phyllosoma of *Panulirus japonicus* (Matsuda, 2005).

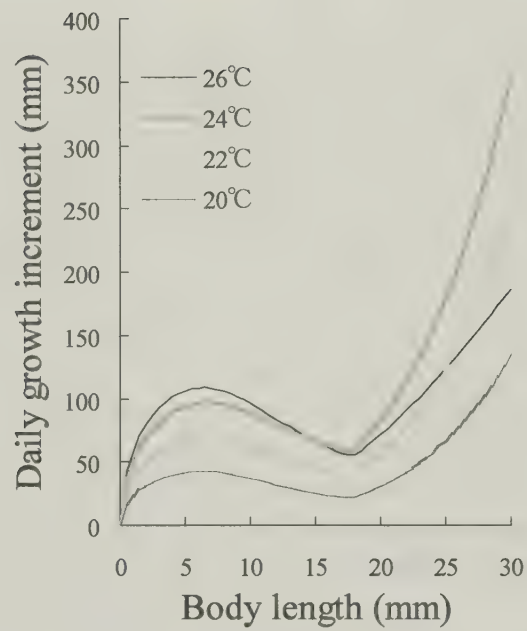


Fig. 3. Effect of temperature on daily growth increment, as calculated from molt increment in body length divided by intermolt period, throughout the entire phyllosoma phase of *Panulirus japonicus* (Matsuda and Yamakawa, 1997).

temperature in the early stage phyllosoma (Matsuda, 2005). Growth efficiency increased as temperature increased. On the assumption that the temperature that gives the highest efficiency is optimal, 26°C is the optimal temperature for these early stages.

Matsuda and Yamakawa (1997) examined the effect of temperature on daily growth increment (DGI), calculated from the molt increment in BL divided by the intermolt period, throughout the entire phyllosoma phase. Smaller larvae (<ca. 15 mm) showed the largest DGI at 26°C; for larvae that were around 15 mm long the 26 and 24°C lines were very close together; larger larvae showed the largest DGI at 24°C (Fig. 3). Larval mortality rates showed a trend similar to that of DGI: survival rate decreased as temperature increased for larger larvae (Matsuda and Yamakawa, 1997). These results indicate that the optimal temperature is 26°C for larvae smaller than around 15 mm BL and 24°C for larger larvae.

Light

Photoperiod and light intensity are also important in culturing phyllosoma because they affect behavior and physiological conditions and, in turn, growth and survival. When phyllosoma are cultured under a natural light-dark cycle they molt synchronously at around dawn, except during a period of about a month after hatching (Fig. 4); the timing of molting is regulated by an endogenous rhythm that is induced by the light-dark cycle (Matsuda *et al.*, 2003). This suggests that a stable light-dark cycle is important for culturing *P. japonicus* phyllosoma,

because a sudden change in photoperiod may disturb the molting rhythm of the larvae, as has been seen in a scyllarid lobster (Mikami, 2005). However, phyllosoma cultured under light phases of three lengths (10, 12, and 18 h) in a day showed no significant differences in growth and survival (Matsuda *et al.*, 2003).

Newly hatched larvae of *P. japonicus* display a strongly photopositive reaction. This photopositive reaction gradually disappears with development, and beyond 4 to 5 mm BL they generally show a photonegative reaction (Saisho, 1966). These positive and negative reactions to light lead to aggregation and tangling of the larvae when they are cultured communally in tanks. The tangling can cause loss of long maxillipeds and pereopods, resulting in slow growth and low survival rates. To avoid tangling, the phyllosoma of some palinurid lobsters have been cultured at low light intensities or in the dark (Moss *et al.*, 1999, Matsuda and Takenouchi, 2005). Low light intensities also help maintain an even distribution of food (*Artemia*) in the tanks so that no behavioral separation between larvae and food occurs (Moss *et al.*, 1999). Stage I phyllosoma of *J. edwardsii* reared at a photon flux density of $0.001 \mu \text{mol s}^{-1} \text{m}^{-2}$ or in the dark grew larger than those reared at 0.1 or $10 \mu \text{mol s}^{-1} \text{m}^{-2}$.

Culture tank

In many studies of *P. japonicus* phyllosoma culture, cylindrical tanks with concave or flat bottoms were used, but rates of survival to the puerulus stage were low, at less than 10% (Inoue, 1981; Kittaka,

2000; Sekine *et al.*, 2000). Newly designed tanks were recently introduced to phyllosoma culture, and survival rates have been gradually increasing. Matsuda and Takenouchi (2005) designed a 40-L culture tank and obtained high survival rates of 37% to 54% to the puerulus stage from the middle phyllosoma stage (mean BL, 11.5 mm). This tank is elliptical and shallow, with a concave bottom and smoothly curved corners; it was designed to prevent excessive aggregation of larvae and to allow the larvae to be conveniently observed (Fig. 5). Takushi Horita of the Toba Aquarium introduced to phyllosoma culture a tank with a modified upwelling system (called the “planktonkreisel” and originally developed by Greve in 1975 for the maintenance of planktonic animals), and produced several pueruli

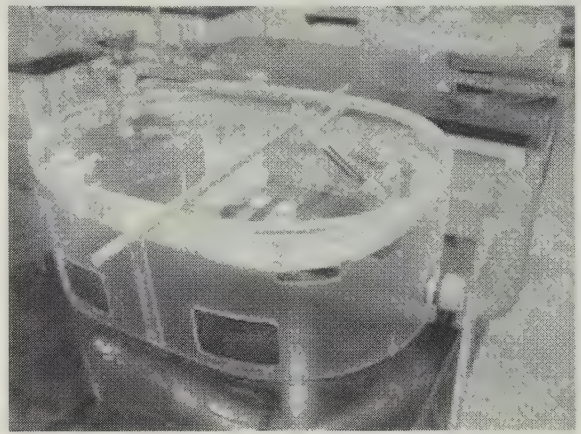


Fig. 5. Culture tank for *Panulirus japonicus* phyllosoma used at the Fisheries Research Division, Mie Prefectural Science and Technology Promotion Center (Matsuda and Takunouchi, 2005).

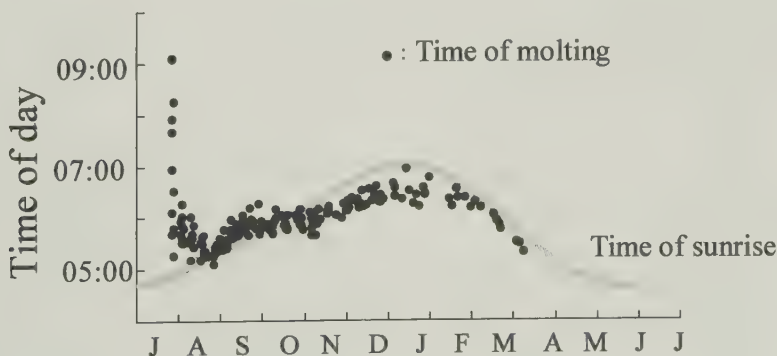


Fig. 4. Diel timing of molting of *Panulirus japonicus* phyllosoma cultured under natural light-dark cycle (Matsuda *et al.*, 2003).

(Horita, T., personal communication) (Fig. 6). A constant circular current is provided in the tank by water input at the top; the current keeps the larvae suspended in the water column and prevents them from aggregating and tangling with each other. Murakami (2004) further improved the upwelling system and designed a rotatory tank with a capacity of 70 L; the tank is placed in a large water bath and rotates by itself to provide a more moderate circular current (Fig. 7). His attempt was successful, and he obtained a survival rate of 28.3% to the juvenile stage from hatching.

Table 2 lists the types of phyllosoma culture tanks that have so far been used for palinurid lobsters; they can be classified into three types on

the basis of their configurations: horizontal, vertical, and intermediate. The horizontal type has the advantages of enabling easy operation of the culture systems and convenient observation of larvae. However, it also has disadvantages in that the larvae are always in contact with the bottom of the tank, and this appears to cause diseases and mortality. Moreover, it is difficult to scale up the larval culture because these types of tank need large amounts of space. The vertical type has advantages in that it is possible to maintain the larvae in suspension with a moderate vertical water current and it is easier to scale up the larval culture because the tanks do not need large amounts of space. However, the culture systems used with this type of tank are generally

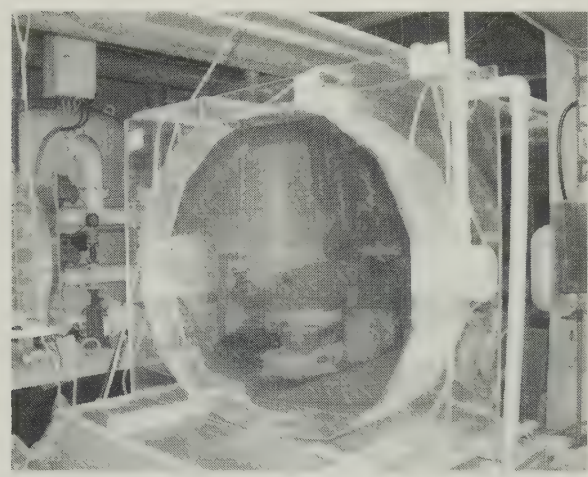


Fig. 6. Culture tank for *Panulirus japonicus* phyllosoma used at Toba Aquarium (Courtesy of T. Horita).

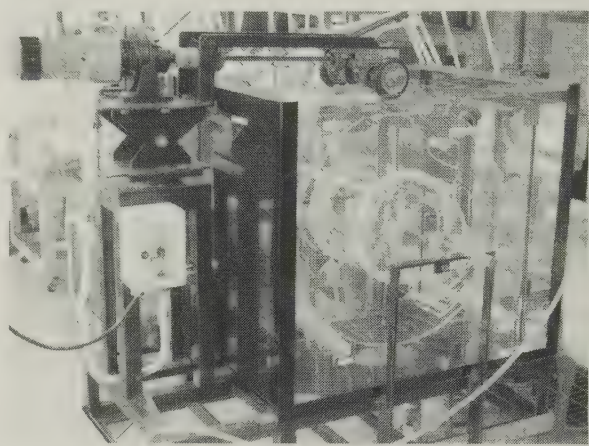


Fig. 7. Culture tank for *Panulirus japonicus* phyllosoma used at Minamiizu Station, National Center for Stock Enhancement, Fisheries Research Agency, Japan (Murakami, 2004).

Table 2. Culture tanks for palinurid phyllosoma

Tank type	Shape of tank	Volume (L)	Species	Reference
Horizontal	Elliptical	40	<i>Panulirus japonicus</i>	Matsuda and Takenouchi (2005)
	Circular	30	<i>Jasus edwardsii</i>	Ritar (2001)
Vertical	Square tube	72	<i>Jasus edwardsii</i>	Illingworth <i>et al.</i> (1997)
	Drum	90	<i>Panulirus japonicus</i>	Horita (pers. comm.)
	Drum	70	<i>Panulirus japonicus</i>	Murakami (2004)
Intermediate	Cylinder	16-100	<i>Panulirus japonicus</i>	Kittaka (2000)
	Cylinder	40	<i>Panulirus japonicus</i>	Sekine <i>et al.</i> (2000)

complicated, and their operation and management need more attention than those used with horizontal types. Intermediate-type cylindrical tanks have been used in many studies to examine the optimal conditions for flow-through culture of palinurid phyllosoma (*e.g.*, Inoue, 1981; Kittaka, 2000). Such studies have been used as a basis for the development of culture tanks, but this type of tank has disadvantages in that the larvae huddle together in a small area because the tank generally has a small bottom, and it is difficult to provide a moderate water current. At present, it is difficult to decide on which type is best for scaling up phyllosoma culture; further study of tank design will be required for the establishment of large-scale phyllosoma culture.

Future work

Culturing techniques for *P. japonicus* phyllosoma have been improving step by step; these improvements now enable us to produce more than 100 juveniles a year through phyllosoma culture. However, there are many problems to be solved in establishing large-scale phyllosoma culture, including the need to reduce the use of antibiotics in preventing bacterial diseases and the need to increase the efficiency of the culture procedure. The prevention of bacterial diseases is of paramount importance in larval culture. Hereafter, various new pathological and nutritional approaches and new culture techniques are needed.

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BULLETIN OF FISHERIES RESEARCH AGENCY

No. **20**
February 2007

Bull.Fish.Res.Agen.

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November 24-25, 2005

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